The effect of phosphodiesterase type5 inhibitors on the development of retinopathy of prematurity: A Randomized Clinical Trial

Running title: Effect of sildenafil on the development of ROP

Abstract

Background: Retinopathy of prematurity (ROP) which affecting premature infants characterized by the development of vascular proliferation due to hyperoxia, down regulation of VEGF and death of endothelial cells. We hypothesized that inhibition of Phosphodiesterase 5 enzyme suppress retinal vasoconstriction and prevent ROP.

Study design: 109 newborns with respiratory distress syndrome treated with oxygen with early gestational age (GA) ≤30 weeks and birth weight (BW) ≤1500g were randomized into two groups, Group sildenafil (as cases group) and placebo Group (as control group), sildenafil were administered via nasogastric tube. Occurrence of ROP phase 1 as primary outcome and stage 2-5 ROP, duration of mechanical ventilation, NCPAP, oxygen therapy and duration of hospitalization as secondary outcomes were assessed.

Result: 52 patients in sildenafil and 20 patients in placebo group were studied. There were no differences between the two groups in demographic characteristics. ROP phase 1 was seen in 11(22%) and 7(14%) of placebo and interventional group, respectively. Stage 3 ROP was not seen in any of the patients

Conclusion: Sildenafil therapy did not affect ROP development in premature infants treated with oxygen. May be due to our exclusion criteria (BW less than 1000g) and this fact that high incidence of ROP is in extremely low birth weight neonates, we didn't find any significant differences. More studies with larger population and expanded criteria are needed to find the effect of sildenafil on ROP.

Key Words: Retinopathy of Prematurity; Premature infants; Sildenafil; Oxygen therapy; Respiratory Distress Syndrome

UNDER PEER REVIEW

Introduction

Visual impairment classified at 4 levels of visual function according to the WHO definition includes: normal vision, moderate visual impairment, severe visual impairment, and blindness. The term "low vision" refers to moderate and severe visual impairment (1).

Retinopathy of prematurity (ROP) is a leading cause of childhood blindness worldwide is characterized by the development of vascular proliferation due to hyperoxia causing down regulation of VEGF and death of endothelial cells (2-4). Premature retinopathy is a biphasic condition comprising an initial phase of vessel loss followed by a second phase of vessel proliferation(5). It is believed that this process is responsible for the relative hyperoxia of the extra-uterine environment as well as the additional oxygen given to premature infants. Regularly in utero PaO2 is 30 mm Hg and the blood is only ~70 percent saturated as opposed to 100 percent full-term newborns in room air with 60–100 mm Hg PaO2 (5, 6). The non-vascularized retina turns out to be progressively metabolically active as the newborn child develops and leads to tissue hypoxia without a sufficient vascular framework. The first phase of ROP occurs about 30–32 weeks from birth to postmenstrual age. The second phase is retinal neovascularization induced by hypoxia and begins around the postmenstrual age of 32–34 weeks(7).

As premature births increase and survival rates improve in view of advances in neonatal consideration, the number of infants at risk for ROP has been expanding around the world, particularly in middle-income countries(8) The incidence of ROP is different from country to country depending on the economy and social conditions, in 2010, an expected 184,700 babies of 14.9 million premature babies developed any phase of ROP; 20,000 of them became blind or severely visually impaired from ROP(3).

ROP is a multifactorial disease and different studies report several risk factors associated with this condition, some of which can cause severe ROP including, early gestational age (GA) at ≤30 weeks, low birth weight (BW) at ≤1500g, supplemental oxygen, prolonged mechanical ventilation, Apgar score, pulmonary complications, anemia, interventricular hemorrhage (IVH), necrotizing enterocolitis and sepsis (9-11)

The transcription factors HIF-1α (Hypoxia-Inducible Factor) (HIF), HLF (HIF-1α-like factor, also known as EPAS1) and HIF-2α play important roles in the body's response to low oxygen concentrations AND embryonic vascularization plays an integral role and one the most important of its function during hypoxia is to promote angiogenesis by regulation of expression of genes such as vascular endothelial growth factor (VEGF) (12).

Although conflicting reports on the effects of phosphodiesterase inhibitors (PDEs), Phosphodiesterase type 5 inhibitors (PDE5-Is) have a potential therapeutic strategy for different disorder such as, neurodegenerative diseases and ROP(13). The PDE superfamily consists of 11 subtypes (PDE1–PDE11)(14). PDE5 is an enzyme strongly expressed in cerebellum, When PDE5 is inhibited the vasodilatory effect of NO is enhanced(13). Expression of elevated HIF1α exerts proangiogenic effects through several downstream effectors, including VEGF. Regulating the expression of HIF1α through PDE5 inhibition could have a beneficial vasoprotective effect on ROP(15). In this clinical trial study we assess the effect of sildenafil, a PDE5 inhibitor, on the development of phase 1 ROP as primary effect and stage 2-5 ROP, duration of mechanical

UNDER PEER REVIEW

ventilation, NCPAP, oxygen therapy and duration of hospitalization as secondary outcomes. We hypothesized that Phase 1 retinopathy and thereby phase 2 ROP can be suppressed by preventing degradation of HIF-1 and VEGF.

Material and methods

Study design and participants

A total of 109 subject have been enrolled in this randomized, double-blind, placebo-controlled clinical trial at Imam Khomeini Hospital's Neonatal Intensive Care Unit, Ahvaz Jundishapur Medical Science University, Ahvaz, IRAN, from March 2014 through December 2015. An informed consent was obtained from patients' parents. In this study based on our previous study(16) and the incidence of stage 3 ROP (8 percent), considering 80 percent as the study's power and 95 percent confidence interval, 50 patients were enrolled in each group. In this investigation, infants with birth body weight below 1500 g and more than 1000 g with breathing distress were qualified. Given the high death rate of infants weighing less than 1000 g at birth. Also excluded were 150 mg percent blood sugar for more than 7 days, pH<7.2 for more than 24 hours and 10ml / kg blood transfusion for the first four weeks of life.

Informed consent and ethics committee approval

This study was approved by Ahvaz Jundishapur University of Medical Sciences ethics committee (AJUMS.REC.1393.405). The trial was also registered in the Iranian Registry of Clinical Trials with registration number IRCT2015102314215N3. The informed written consent was obtained from each patient.

Randomization, Blinding, data recording and Intervention

ROP screening was performed by an expert ophthalmologist on the basis of International Classification of Premature Retinopathy Revisited (ICROPR). The same ophthalmologist followed the patients until the 45th post-conceptual age. The doctor and caretaker were blinded to the vial content and the patients were enrolled according to the computerized randomization list table in the study. Surfactant doses; blood volume transfusion; analyzes of Arterial Blood Gas (ABG) numbers; duration of M.V, NCPAP and oxygen therapy; blood sugar; and doses of antenatal betamethasone were recorded in all babies with respiratory distress at 6 cm H2O. Children were treated with 200mg / kg surfactant (survanta) when the requirements for Fio2 were 40 %. Technique for surfactant therapy INSURE (INtubate–SURfactant–Extubate to

UNDER PEER REVIEW

CPAP)(17). Mechanical ventilation (MV) was considered in babies with PaO2 < 50 mmHg or PaCO2 > 55 mmHg and pH < 7.25 while being treated with FiO2 > 0.4 and PEEP > 6 cm H2o; or those with increased breathing work including severe intercostal retractions on PEEP > 7 cm H2o; or prolonged (> 20 s) or recurrent apneas and bradycardia (> 2 episodes within 24 h) need bag and mask ventilation (18, 19). In newborns with respiratory distress, additional doses of surfactant were administered while being treated with NCPAP or M.V and requiring a concentration of oxygen of about 40% (17). Ventilated newborns with appropriate ABG (Pao2 60–80 mmHg, Paco2 40–55 mmHg and pH 7.25–7.45) and without increasing breathing work were moved to NCPAP when they received low PIP (10–12 cm H2o), less than 40 percentFio2 and 10–15/min breathing rates (20). Based on the computerized randomization list, placebo (control group) or Sildenafil (interventional group) were given in each patient group. In the same volume and color with clinical pharmacist, a solution containing Sildenafil 1 mg / ml or placebo was prepared. Placebo and Sildenafil solution vials were marked with A and B, respectively. A volume equal to 1 ml / kg of solution (solution A or B) was given every 8 hours in each patient group. Through a nasogasteric tube. The nasogasteric tube was subsequently washed with distilled water. During oxygen therapy, sildenafil or placebo was administered.

Statistical analysis

Comparison between continuous and independent variables was performed using Mann–Whitney, and chi-square test. All the statistical analysis was performed using SPSS version 16 (IBM, Armonk, New York). P Value <0.05 was considered significant.

Results

Figure 1 shows the flow diagram of this trial. The study was completed by a total of 102 subjects. At the baseline, the sildenafil group (n=56) and placebo group (n=53) were randomly assigned to 109 participants. Of the 109 participants, 4 were from the group arm of sildenafil and 3 were dropped from the group of placebo. (Fig. 1).

UNDER PEER REVIEW

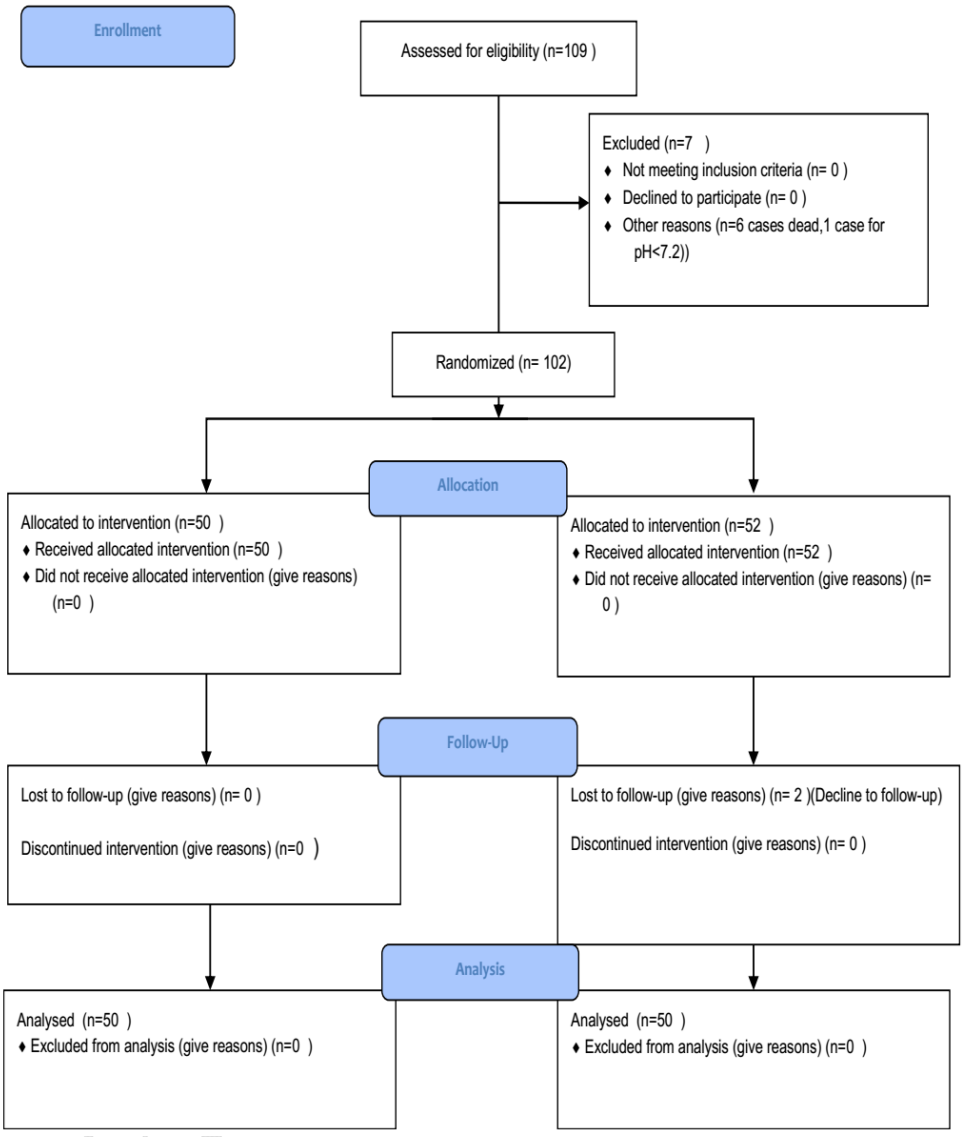

Fig. 1. Flowchart showing recruitment of participants, randomization and completion.

Effects of sildenafil treatment on ROP outcome

Table I presents detailed demographic and morbidity information by sildenafil treatment. There were no differences between the two groups in demographic characteristics (P > .05).

Table1.Demographic data and morbidities in cases and controls Characteristics. Sildenafil-treated (cases; n = 52) placebo (controls; n = 50).

characteristics	**Sildenafil-treated (cases; n = 52)**	**placebo (controls; n = 50)**	***P* value***
Birth weight, g, mean ± SD; median (range)	1257± 150	1285± 142.7	0.338
Gestational age, wk, mean ± SD;	27.17±1.94	28.19±1.82	0.959

median (range)			
Cesarean delivery, n (%)	32(62)	29(58)	0.789
Male sex, n (%)	22(42)	27(54)	0.624
Five-min Apgar score <7, n (%)	23(44)	24(48)	0.992
Receipt of postnatal steroids, n (%)	19(36)	17(34)	0.665
Patent ductus arteriosus requiring treatment, n (%)	36(69)	32(64)	0.552
Grade III or IV intraventricular hemorrhage, n (%)	5(9)	4(8)	0.423
Necrotizing enterocolitis, n (%)	6(11)	5(10)	0.687
Receipt of red blood cell transfusion, n (%	42(80)	40(50)	0.774

Stage 1 and 2 ROP was seen in 11(22%) and 7(14%) of placebo and sildenafil groups respectively. Stage 3 ROP was not seen in any of the patients. There were no differences between groups in clinical course (Table2).

Patients with zone I retinopathy of ROP have poor outcomes despite treatment. We analyze the frequency of zone I, II and III in patients treated with sildenafil or placebo. In placebo group 2 patients were in Zon1 and in intervention group no case was in Zon1 (Table3). From total patients that were in (Zon1+Zon2): 8 patients (73%) were in placebo group and 2 patients (27%) were in interventions group. Affection of Zon3 in sildenafil group was 5 patients (71.5%) and in control group was 3 patients (28.5%) that there were no significantly differences in two groups. The number of Arterial Blood Gas (ABG) sampling were not different between two groups.

Table 2.Frequency of severe ROP in sildenafil and placebo groups. Sildenafil-treated (cases; n = 52) placebo (controls; n = 50).

	Placebo (n, %)	Sildenafil (n, %)	Total (n, %)
Stage 1 and 2 ROP	11(22)	7(14)	18(17)

Table 3. The frequency of zone I, II and III in patients treated with sildenafil or placebo

		group		Total
		Placebo (n, %)	Sildenafil (n, %)	
Zone	1	2(18)	0(0)	2
	2	6(55)	2(28.5)	8
	3	3(27)	5(71.5)	8
Total		11(100)	7(100)	18(100)

UNDER PEER REVIEW

Discussion

Despite current late-stage surgical treatment, premature retinopathy (ROP) is still a major cause of worldwide blindness in premature infants (21).). In the developing and developed world, there are at least 50 000 blind children from ROP worldwide, which remains an important cause of childhood blindness (1, 2).

During the 1990s, significant advances in ROP treatment came when cryotherapy and laser photocoagulation of avascular retina appeared to be mostly successful in counteracting visual impairment in newborn children with ROP. Although these therapies may decrease the rate of visual impairment by 25 percent in late-organized babies, the patients still have poor visual acuity after treatment on a regular basis. Preventive and less harmful treatments for ROP would be much more attractive, and understanding of ROP's molecular mechanisms is essential for improving such medicinal interventions (5).

It is hypothesized that if the amount of production of HIF-1α does not reduce in the body after birth and oxygen therapy, it can be prevent the development of ROP in preterm infants. Phosphodiesterase-inhibiting (PDE-5) drugs by inhibiting cGMP hydrolysis increase the production of HIF-1α and subsequently increase VEGF and accelerate angiogenesis. Sildenafil is reversible and potent PDE5 inhibitor that effectively inhibits cGMP hydrolysis (22). In this investigation we evaluate the developing of ROP in preterm infants in south-west of Iran.

In present study ROP developed in 18% of patients, 7(14%) and 11(22%) of control and sildenafil groups, respectively. However the differences between two groups was not significant, but ROP developed lesser in sildenafil group. Fawzi et al showed that in a mouse OIR model, Sildenafil significantly reduced retinal vaso-obliteration and neovascularization (15). In previous our study the incidence of stage 3 ROP was 8%, while in this present study stage 3 ROP were not seen in any of the studied cases. Thus we did not able to assess the effect of sildenafil on the progression of stage 1 ROP toward stage 3-5 ROP(16).

Yassen et al in a study in 2012 showed sildenafil Enhanced oxygenation and reduced mortality without an important clinical complication in infants with pulmonary arterial hypertension(23). Marsh and colleagues in 2004 reported a 26-wk baby was treated with sildenafil. Sildenafil for 16 days were used and patient was randomly assigned to a ROP until 31 wk had no ROP. At 34wk, he was afflicted to ROP Stage 3(24). Kehat et al., in 2010, studied 22 neonates with a gestational age of more than 34 weeks and a weight of more than 2100 grams that received more than 2 weeks of sildenafil and were evaluated by the pediatric

UNDER PEER REVIEW

ophthalmologist for possible side effects. They concluded that babies who have received sildenafil do not need a routine ophthalmologic examination(25).

Through the past 4 year's relative improvement of neonatal intensive care and monitoring of oxygen therapy result in decreasing incidence of ROP in our center. However neonatal intensive care in our center is still suboptimal. So the number of Arterial Blood Gas sampling was low in our patients and monitoring of oxygen therapy were substantially depended on pulse oximetry. Sildenafil did not effect on the duration of mechanical ventilation, NCPAP, oxygen therapy and hospitalization. Sildenafil improved survival and echocardiographic finding of persistent pulmonary hypertension in term newborn(26, 27) but does not improve oxygenation during ARDS(28). Because of high incidence of ROP in extremely low birth weight neonates (less than 1000g) exclusion of them was the major limitation of our study. In conclusion, this study shows that sildenafil administration did not significantly affect the incidence of ROP in premature infants treated with oxygen.

Conflict of interest: The authors declare that they have no conflict of interests.

References

1. Kong L, Fry M, Al-Samarraie M, Gilbert C, Steinkuller PG. An update on progress and the changing epidemiology of causes of childhood blindness worldwide. Journal of American Association for Pediatric Ophthalmology and Strabismus. 2012;16(6):501-7.

2. Gilbert C. Retinopathy of prematurity: a global perspective of the epidemics, population of babies at risk and implications for control. Early human development. 2008;84(2):77-82.

3. Blencowe H, Lawn JE, Vazquez T, Fielder A, Gilbert C. Preterm-associated visual impairment and estimates of retinopathy of prematurity at regional and global levels for 2010. Pediatric research. 2013;74(S1):35.

4. Kim SJ, Port AD, Swan R, Campbell JP, Chan RP, Chiang MF. Retinopathy of prematurity: a review of risk factors and their clinical significance. Survey of ophthalmology. 2018;63(5):618-37.

5. Chen J, Smith LE. Retinopathy of prematurity. Angiogenesis. 2007;10(2):133-40.

6. Bell E, Klein J. Comments on oxygen toxicity and retinopathy (ROP) in the premature infant. Iowa neonatology handbook: pulmonary (University of Iowa, Children's hospital, Department of Pediatrics). 1994.

7. Chen J, Stahl A, Hellstrom A, Smith LE. Current update on retinopathy of prematurity: screening and treatment. Current opinion in pediatrics. 2011;23(2):173.

UNDER PEER REVIEW

8. Mariotti A, Pascolini D. Global estimates of visual impairment. Br J Ophthalmol. 2012;96(5):614-8.

9. Celebi ARC, Petricli IS, Hekimoglu E, Demirel N, Bas AY. The incidence and risk factors of severe retinopathy of prematurity in extremely low birth weight infants in Turkey. Medical science monitor: international medical journal of experimental and clinical research. 2014;20:1647.

10. Darlow BA, Hutchinson JL, Henderson-Smart DJ, Donoghue DA, Simpson JM, Evans NJ. Prenatal risk factors for severe retinopathy of prematurity among very preterm infants of the Australian and New Zealand Neonatal Network. Pediatrics-English Edition. 2005;115(4):990-6.

11. Eckert G, Fortes Filho J, Maia M, Procianoy R. A predictive score for retinopathy of prematurity in very low birth weight preterm infants. Eye. 2012;26(3):400.

12. Ziello JE, Jovin IS, Huang Y. Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. The Yale journal of biology and medicine. 2007;80(2):51.

13. Peixoto CA, Nunes AKS, Garcia-Osta A. Phosphodiesterase-5 inhibitors: action on the signaling pathways of neuroinflammation, neurodegeneration, and cognition. Mediators of inflammation. 2015;2015.

14. Kotera J, Fujishige K, Omori K. Immunohistochemical localization of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in rat tissues. Journal of Histochemistry & Cytochemistry. 2000;48(5):685-93.

15. Fawzi AA, Chou JC, Kim GA, Rollins SD, Taylor JM, Farrow KN. Sildenafil attenuates vaso-obliteration and neovascularization in a mouse model of retinopathy of prematurity. Investigative ophthalmology & visual science. 2014;55(3):1493-501.

16. Feghhi M, Altayeb SMH, Haghi F, Kasiri A, Farahi F, Dehdashtyan M, et al. Incidence of retinopathy of prematurity and risk factors in the south-western region of Iran. Middle East African journal of ophthalmology. 2012;19(1):101.

17. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, et al. European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants-2013 update. Neonatology. 2013;103(4):353-68.

18. Kugelman A, Feferkorn I, Riskin A, Chistyakov I, Kaufman B, Bader D. Nasal intermittent mandatory ventilation versus nasal continuous positive airway pressure for respiratory distress syndrome: a randomized, controlled, prospective study. The Journal of pediatrics. 2007;150(5):521-6. e1.

19. Urs PS, Khan F, Maiya P. Bubble CPAP-a primary respiratory support for respiratory distress syndrome in newborns. Indian pediatrics. 2009;46(5).

UNDER PEER REVIEW

20. Spitzer AR, Clark RH. Positive-pressure ventilation in the treatment of neonatal lung disease. Assisted ventilation of the neonate: Elsevier; 2011. p. 163-85.

21. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010;126(3):443.

22. Nehra A, Colreavy F, Khandheria B, Chandrasekaran K. Sildenafil citrate, a selective phosphodiesterase type 5 inhibitor: urologic and cardiovascular implications. World journal of urology. 2001;19(1):40-5.

23. Yaseen H, Darwich M, Hamdy H. Is sildenafil an effective therapy in the management of persistent pulmonary hypertension? Journal of clinical neonatology. 2012;1(4):171.

24. Marsh C, Marden B, Newsom R. Severe retinopathy of prematurity (ROP) in a premature baby treated with sildenafil acetate (Viagra) for pulmonary hypertension. British journal of ophthalmology. 2004;88(2):306-7.

25. Kehat R, Bonsall DJ, North R, Connors B. Ocular findings of oral sildenafil use in term and near-term neonates. Journal of American Association for Pediatric Ophthalmology and Strabismus. 2010;14(2):159-62.

26. Baquero H, Soliz A, Neira F, Venegas ME, Sola A. Oral sildenafil in infants with persistent pulmonary hypertension of the newborn: a pilot randomized blinded study. Pediatrics. 2006;117(4):1077-83.

27. Mourani PM, Sontag MK, Ivy DD, Abman SH. Effects of long-term sildenafil treatment for pulmonary hypertension in infants with chronic lung disease. The Journal of pediatrics. 2009;154(3):379-84. e2.

28. Ñamendys-Silva SA, Hernández-Garay M, Rivero-Sigarroa E. Should we administer sildenafil to patients with acute respiratory distress syndrome? No. Intensive care medicine. 2010;36(6):1102-3.

UNDER PEER REVIEW