The effect of phosphodiesterase type5 inhibitors on the development of retinopathy of prematurity in Imam Khomeini Hospital's, Ahvaz, Iran preterm infants: A Randomized Clinical Trial

Running title: Effect of sildenafil on the development of Retinopathy of prematurity

List of abbreviation: Acute respiratory distress syndrome (ARDS), Arterial Blood Gas (ABG), birth weight (BW), chronic obstructive pulmonary disease (COPD), Continuous positive airway pressure (CPAP), Fraction of inspired oxygen (FiO2), gestational age (GA), HIF□1α□like factor (HLF), Hypoxia-Inducible Factor (HIF), International Classification of Premature Retinopathy Revisited (ICROPR), INtubate–SURfactant–Extubate (INSURE), Mechanical ventilation (MV), Millimeter of mercury(mmHg), Partial Pressure of Oxygen (PaO2), Nasal continuous positive airway pressure (NCPAP), phosphodiesterase inhibitors (PDEs), Phosphodiesterase type 5 inhibitors (PDE5-Is), Positive end-expiratory pressure (PEEP), Pulmonary Hypertension (PH), Retinopathy of prematurity (ROP), Statistical Package for the Social Sciences version (SPSS), Vascular endothelial growth factor (VEGF).

Abstract

Background: Retinopathy of prematurity (ROP) affects premature infants, and it is characterized by the development of vascular proliferation due to hyperoxia, down regulation of Vascular endothelial growth factor(VEGF) and death of endothelial cells. We hypothesized that inhibition of Phosphodiesterase 5 enzyme suppresses 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, 52 patients in sildenafil and 50 patients in placebo group were studied, Group sildenafil (as case group) and placebo Group (as control group), sildenafil was administered via nasogastric tube. Occurrence of ROP phase 1 as primary outcome and stage 2-5 ROP, duration of mechanical ventilation, oxygen therapy and duration of hospitalization as secondary outcomes were assessed.

Result: There was no differences between the two group 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 there is a high incidence of ROP in extremely low birth weight neonates, we didn't find any

significant difference. 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

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, and it is characterized by the development of vascular proliferation due to hyperoxia causing down regulation of VEGF and death of endothelial cells (2-4).

The International Classification of Retinopathy Prematurity (ICROP) through the collaboration of experts from different countries was first developed in 1984 and later updated in 1987 and 2005 to facilitate a standardized the clinical finding of ROP(5). The elements identified consist of the location (zone), the severity (stage), extent of the abnormal peripheral vascularization, and the presence or absence of plus disease(6). The highest stage and the lowest zone determines the status of ROP. The ROP located in Zone 1 which Zone I is the small circle of retina around the optic disc has the worst prognosis, whereas Zone III which is a crescent-shaped area of temporal retina will in general be mild(6). The stages of ROP are scaled from Stage 1 ROP to Stage 5 ROP five. Stage 1 is marked by the presence of a demarcation line between the normally vascularized retina and the peripheral retina in which there are no blood vessels. Stage 2 is characterized the demarcation line develops into a ridge, with height and width, between the vascular retina and peripheral retina. Stage 3 consists of a ridge and Blood vessels grow and proliferate and are visible in the ridge. In Stage 4, there is a subtotal retinal detachment Vitreoretinal surgery may be indicated and in Stage 5 a total retinal detachment and No treatment is usually possible(7). The aggressive posterior ROP (AP -ROP) was added to ICROP in 2005. This particularly aggressive form of ROP was observed with increasing frequency in the smallest premature neonates(6, 8).

Premature retinopathy is a biphasic condition comprising an initial phase of vessel loss followed by a second phase of vessel proliferation(9). 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 Partial Pressure of Oxygen (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 (9, 10). 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(11).

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(12) 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 (13-15)

The transcription factors HIF-1α (Hypoxia-Inducible Factor) (HIF), HLF (HIF-1α-like factor) 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) (16).

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(17). The PDE superfamily consists of 11 subtypes (PDE1–PDE11)(18). PDE5 is an enzyme strongly expressed in cerebellum, When PDE5 is inhibited the vasodilatory effect of NO is enhanced(17). 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(19). VIAGRA (sildenafil citrate), an oral therapy for erectile dysfunction, is the citrate salt of sildenafil, a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Sildenafil citrate is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate(20). 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 ventilation, Nasal continuous positive airway pressure (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.

Inclusion and exclusion criteria:

In this investigation, babies were all those weighing <1200 g at birth, born in or transferred to, a regional neonatal intensive care unit on the first postnatal day, plus those weighing 1200–1499 g, breathing distress and requiring mechanical ventilation within 24 hours were qualified. Babies were excluded if they had major congenital anomalies, weighing less than 1000 g at birth, 150 mg/dl blood sugar for more than 7 days and 10ml / kg blood transfusion for the first four weeks of life.

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 Mechanical ventilation (MV), 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 INtubate–SURfactant–Extubate (INSURE) to Continuous positive airway pressure (CPAP)(21). Mechanical ventilation was considered in babies with PaO2 < 50 mmHg or PaCO2 > 55 mmHg and pH < 7.25 while being treated with Fraction of inspired oxygen (FiO2) > 0.4 and Positive end-expiratory pressure (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 (22, 23). 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 Millimetre of mercury(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(24). 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 Statistical Package for the Social Sciences version (SPSS) 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).

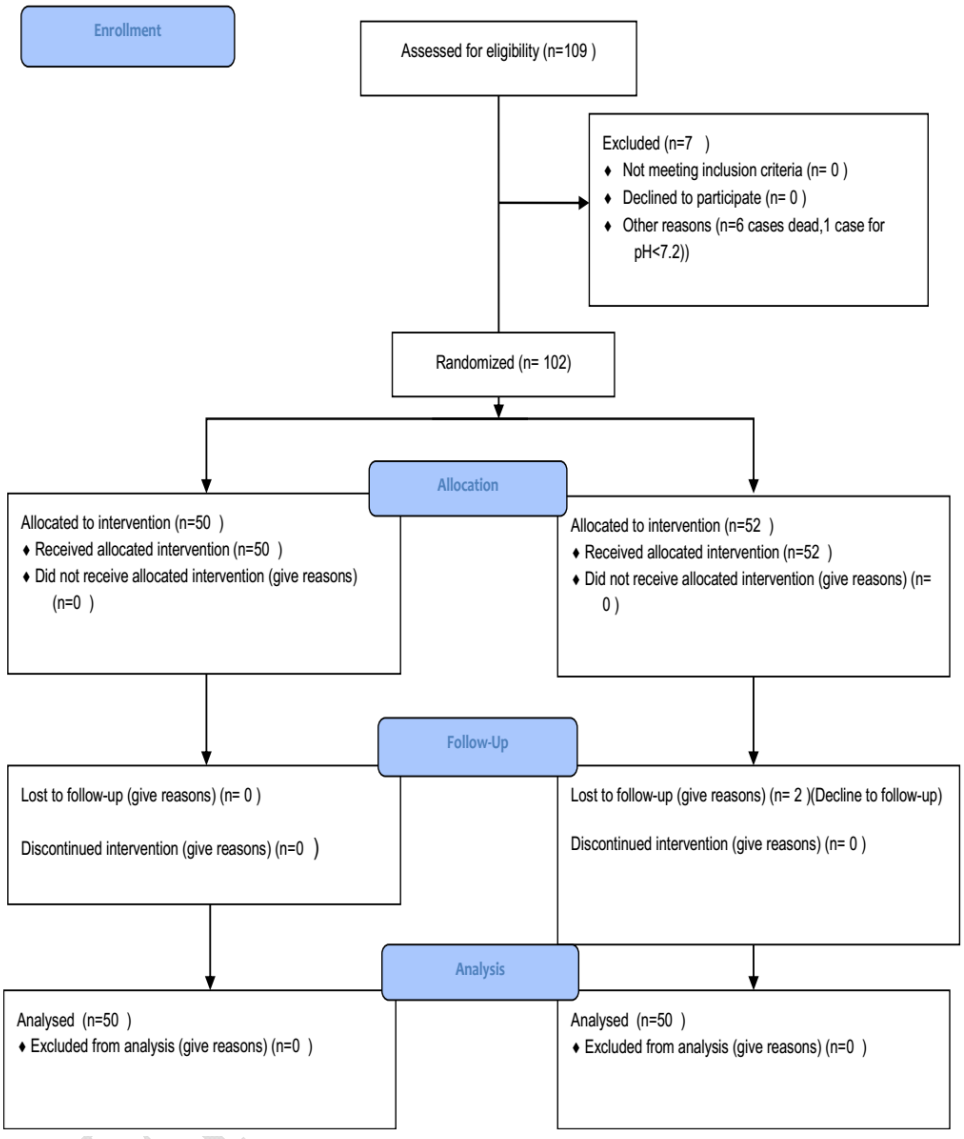

Fig. 1. Flowchart showing recruitment of participants, randomization and completion.

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 Zone1 and in intervention group no case was in Zone1 (Table3). From total patients that were in (Zone1+Zone2): 8 patients (73%) were in placebo group and 2 patients (27%) were in interventions group. Affection of Zone3 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)

Discussion

Despite current late-stage surgical treatment, premature retinopathy is still a major cause of worldwide blindness in premature infants (25).). 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 (9).

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 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 citrate has been shown to oral therapy for erectile dysfunction in a wide range of patients with erectile dysfunction(26) Sildenafil also is able to reduce pulmonary hypertension (PH) which is an important predictor of mortality in chronic obstructive pulmonary disease (COPD)(26). Sildenafil is reversible and potent PDE5 inhibitor that effectively inhibits cGMP hydrolysis (27). 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 (19). In previous study the incidence of stage 3 ROP was 8%, while in this present study stage 3 ROP was not seen in any of the studied cases. Thus we were unable to assess the effect of sildenafil on the progression of stage 1 ROP toward stage 3-5 ROP(28).

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(29). Marsh and colleagues in 2004 reported a 26-wk baby was treated with sildenafil. At 34wk, he was afflicted to ROP Stage 3(30). 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 ophthalmologist for possible side effects. They concluded that babies who have received sildenafil do not need a routine ophthalmologic examination(31).

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(32, 33) but does not improve oxygenation during Acute respiratory distress syndrome (ARDS) (34). 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.

Conclusion:

In conclusion, this study shows that sildenafil administration did not significantly affect the incidence of ROP in premature infants treated with oxygen. Our study has some limitations like as the sample size was small, Perhaps, if the population size was bigger a better result could be observed. We matched the control group as close as possible to the index cases by matching for gestation, birth weight, gender and place of birth. Further work on the retinal effects of sildenafil may be useful in determining whether it truly is a good therapy for preventing of pathogenesis of ROP and Prospective trials may be useful to establish a definite safety profile.

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

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.

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