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Original Article

Aminophylline Infusion Rate Can Affect the Cardiac Rate and Electrocardiographic Findings in Neonate Rats

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ABSTRACT

Background: Cardiac arrhythmias are identified as the major cause of mortality and morbidity in neonates. Most term and preterm infants admitted to the neonatal intensive care unit (NICU) suffer from respiratory diseases, and generally aminophylline as a nonselective phosphodiesterase inhibitor is used. Although aminophylline has several physiological effects on the heart tissue, it has been recognized to have some side effects. Neonates are more prone to its cardiac side effects, notably arrhythmias.

***Objectives*:** This study aimed to evaluate and compare the effects of fast and slow aminophylline injections on electrocardiographic parameters and arrhythmias in neonate rats.

Methods: Thirty-two male Sprague-Dawley rats (10 days old, 50 g) were divided into 4 groups (8 in each): Group I and Group II were treated with 5 mg/kg of normal saline intravenously for 3 and 20 minutes, correspondingly, and Group III and Group IV were treated with a 5-mg/kg bolus of aminophylline intravenously for 3 and 20 minutes, respectively. On the experiment day, the rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) with intraperitoneal injection, and lead II electrocardiograms were recorded. The femoral vein was cannulated using polyethylene catheters (PE50) for the intravenous injection of aminophylline (5 mg/kg) or normal saline.

Results: The rats receiving aminophylline showed a dramatic reduction in the heart rate. Additionally, the PR interval and QTc significantly increased in the rats receiving aminophylline for 3 minutes. Moreover, complete heart blocks, premature ventricular beats, atrioventricular blocks (Mobitz I and Mobitz II), sustained and nonsustained ventricular tachycardias, and AV dissociations were observed.

Conclusions: The results of the current study indicated that a slow infusion rate could prevent the cardiac complications of aminophylline, particularly arrhythmias, in neonate rats. ***(Iranian Heart Journal 2020; 21(4): 22-31)***

KEYWORDS: Aminophylline, Neonate, Heart rate, Cardiac arrhythmia, Rat

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Cardiac arrhythmias constitute the main cause of mortality and morbidity in infants. [1] Arrhythmias, also known as irregular cardiac rhythms or irregular rhythms, are associated with fast or slow heart rates. [2] Bradycardia, or an abnormally slow heart action, is characterized by sinus bradycardia, junctional bradycardia, or atrioventricular (AV) blocks in the pediatric population. [3] Supraventricular tachycardias, as abnormally fast heart rhythms arising from improper electrical activities in the upper part of the heart, comprise atrioventricular re-entrant tachycardias, atrial ectopic tachycardias, and permanent junctional reciprocating tachycardias, which are correlated with fast rhythms. [2] It has been documented that electrocardiographic (ECG) properties in infants and children, due to the age-related changes in anatomy and physiology, are different from those in adults. [4] Bradycardia in children is likely to happen due to hypervagotonia, increased intracranial pressure, and breath-holding. It is also highly related to apnea in premature newborns. [3] In previous research, the incidence of arrhythmias in newborns admitted to the intensive care unit (ICU) was found to be on the rise, and its prevalence was estimated to be from 1% to 5% up to 8.5% in newborns. [5, 6]

The stimulating agents of arrhythmias have been identified as acquired heart diseases such as myocarditis, cardiomyopathies, and electrolyte disturbances. [7-9] Recently, cardiac arrhythmias have been identified as the side effects of noncardiac drugs. [9] Some studies have demonstrated that antiarrhythmic and non-antiarrhythmic drugs predispose neonates and adults to cardiac arrhythmias. [9,10] In effect, drug-induced cardiac arrhythmia-related re-entry has been reported to trigger activities and lead to bradycardia. [10] Most term and preterm infants admitted to the neonatal intensive care unit (NICU) suffer from respiratory diseases. [11] The respiratory morbidity of infants admitted to the NICU, particularly those born before 34-week gestation, is reported to have risen to 44%. [12] In a previous study, the most common diseases in neonates were reported to be respiratory distress syndrome and apnea. [13] Therapeutic protocols for these diseases include continuous positive airway pressure, mechanical ventilation, the consumption of exogenous surfactants through the endotracheal tube, antibiotic therapy, and anti-inflammatory agents or methylxanthines such as aminophylline. [14, 15] Different mechanisms of action such as phosphodiesterase inhibition and adenosine receptor antagonism are recognized for methylxanthines. [16]

Aminophylline is known as a nonselective phosphodiesterase inhibitor used in the treatment of asthma in adults. [17] Apnea has been identified as the most common cause of the admission of preterm neonates to the NICU. [13] In some previous studies, methylxanthines such as aminophylline were suggested for the treatment of apnea. [18] Although aminophylline has several physiological effects on the lung and heart tissues, it has been recognized to have some side effects. [19] As for its cardiac side effects, aminophylline was found to increase the heart rate, stroke volume, cardiac output, and renal blood flow. It was also reported to reduce systemic vascular resistance, arterial pressure, and cerebral blood flow in adults. [19] If the aminophylline level in the blood reaches toxicity, symptoms of toxicity such as anorexia, nausea, vomiting, insomnia, tachycardia, dehydration, and coma appear. [1] Accordingly, we sought to evaluate and compare the effects of aminophylline infusion rates on ECG findings in neonate rats.

METHODS

Study Design

In this study, 32 male Sprague-Dawley rats (10 days old) were purchased from the Animal Reproduction Center of Ahvaz Jundishapur University of Medical Sciences.

The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and was approved by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. IR.AJUMS.REC.1395.120).

Chemicals

Aminophylline was purchased from Darou Pakhsh (Iran), while ketamine HCl (10%) and xylazine (2%) were purchased from Alfasan Co (Woderen -Holland).

Experimental Protocols

The rats were randomly divided into 4 groups: Group I: normal control rats treated with 5 mg/kg of normal saline intravenously for 3 minutes, Group II: normal control rats treated with 5 mg/kg of normal saline intravenously for 20 minutes, Group III: rats treated with a 5-mg/kg bolus of aminophylline intravenously for 3 minutes, and Group IV: rats treated with a 5-mg/kg bolus of aminophylline intravenously for 20 minutes.

Electrocardiographic Recording Method

All the rats were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) with intraperitoneal injection. Fifteen minutes after anesthesia, standard bipolar limb lead II ECG was recorded using a Bio-Amp system and monitored using a PowerLab system (ADInstruments, Australia) so as to evaluate the chronotropic properties. After a 1-minute ECG recording, a longitudinal slot in the groin was created, and the femoral vein was exposed. Then, the femoral vein was cannulated with a polyethylene catheter (PE50) for the injection of aminophylline (5 mg/kg) or normal saline. At the end of the time course (20 min), the incidence rate of premature ventricular beats, AV blocks (first-degree [Mobitz I] and second-degree [Mobitz II] AV blocks), complete heart blocks, and ventricular tachycardias were calculated for the different groups.

Data Analysis

The data analyses were conducted using the SPSS software, version 16. As for the statistical methods, the data in each of the groups were compared using the paired *t*-test. A *P*-value of less than 0.05 was considered significant.

RESULTS

Effects of aminophylline on the heart rate for 3 minutes

The results showed that the heart rates of the neonate rats receiving aminophylline (5 mg/kg) for 3 minutes significantly decreased ($P < 0.001$) (Fig. 1).

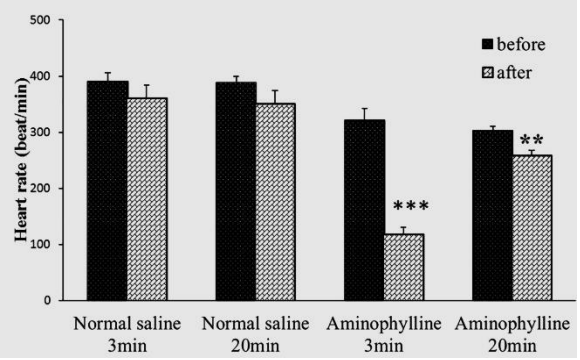


Figure 1: Comparisons are given of the heart rate between the different groups: the control group (normal saline given for 3 min), the control normal group (saline given for 20 min), the aminophylline group (5 mg/kg given for 3 min), and the aminophylline group (5 mg/kg given for 20 min).
***$P < 0.001$, **$P < 0.01$ significant differences before and after treatment (n = 8, mean ± SEM, paired *t*-test)

Effects of aminophylline on the PR interval and Δ heart rates for 3 minutes

The PR interval ($P < 0.001$) (Fig. 2) and Δ heart rates ($P < 0.001$) (Fig. 3) of the neonate rats receiving aminophylline (5 mg/kg) for 3 minutes significantly increased compared with those of the neonate rats treated with saline for 3 minutes.

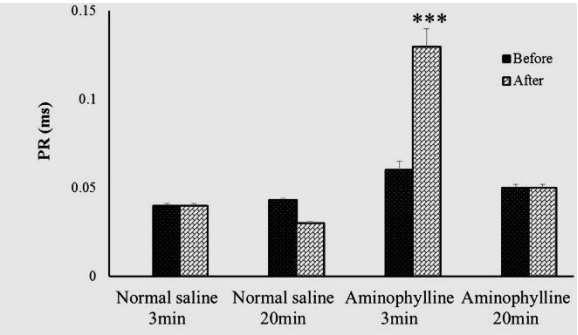


Figure 2: Comparisons are given of the PR interval between the different groups: the control group (normal saline given for 3 min), the control normal group (normal saline given for 20 min), the aminophylline group (5 mg/kg given for 3 min), and the aminophylline group (5 mg/kg given for 20 min). ***P < 0.001, **P < 0.01 significant differences before and after treatment (n = 8, mean ± SEM, paired *t*-test)

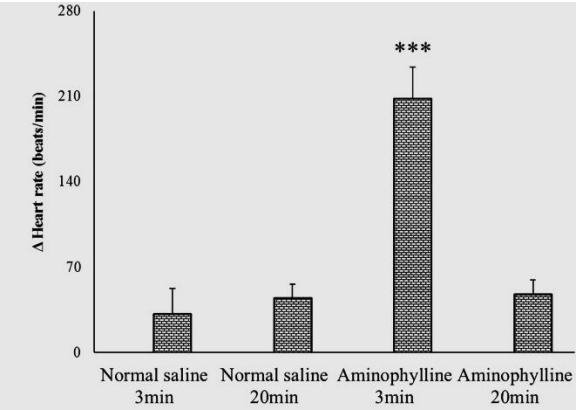


Figure 3: Comparisons are given of the Δ heart rate between the different groups: the control group (normal saline given for 3 min), the control normal group (normal saline given for 20 min), the aminophylline group (5 mg/kg given for 3 min), and the aminophylline group (5 mg/kg given for 20 min). ***P < 0.001, **P < 0.01 significant differences before and after treatment (n = 8, mean ± SEM, paired *t*-test)

Effects of aminophylline on the heart rate for 20 minutes

The neonate rats receiving aminophylline (5 mg/kg) for 20 minutes experienced a significant decrease in the heart rate (P < 0.01) (Fig. 1) compared with the neonate rats that received normal saline for 20 minutes. Nonetheless, no changes were observed in the PR interval of this group (Fig. 2). Their heart rates were also similar to those of the neonates receiving normal saline for 3 and 20 minutes.

Effects of aminophylline on QTc

The neonate rats given aminophylline (5 mg/kg) for 3 minutes showed a significant increase in QTc (P < 0.05) (Fig. 4) compared with the neonate rats receiving aminophylline for 20 minutes along with normal saline.

Arrhythmias found in this study are depicted in Table1 and Figure 5. As can be seen, sustained and nonsustained ventricular tachycardias, along with AV dissociations, were observed.

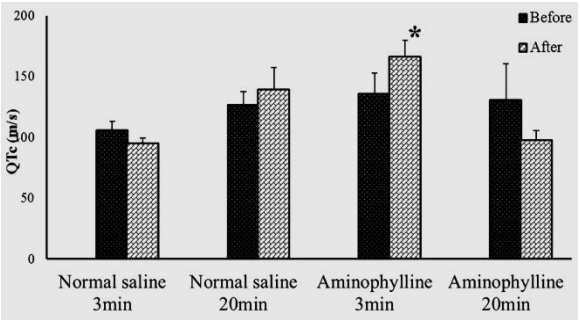
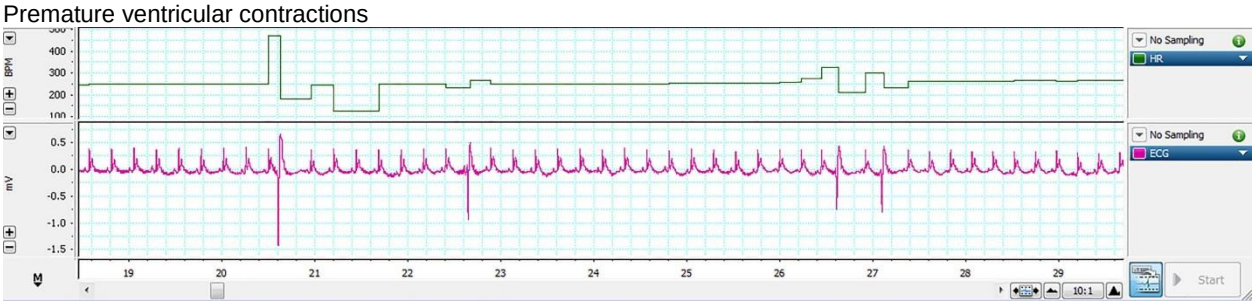


Figure 4: Comparisons are given of QTc between the different groups: the control group (normal saline given for 3 min), the control normal group (normal saline given for 20 min), the aminophylline group (5 mg/kg given for 3 min), and the aminophylline group (5 mg/kg given for 20 min). ***P < 0.001, **P < 0.01 significant differences before and after treatment (n = 8, mean ± SEM, paired *t*-test)

A: Control

B: Aminophylline for 3 min
Premature ventricular contractions

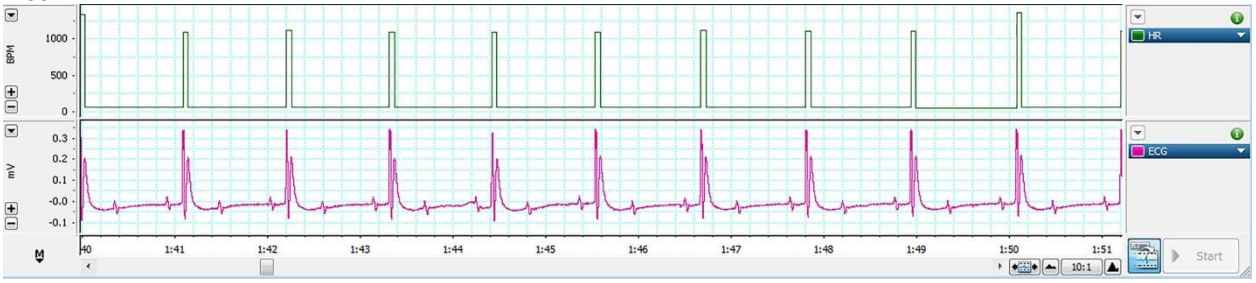


Premature ventricular contractions

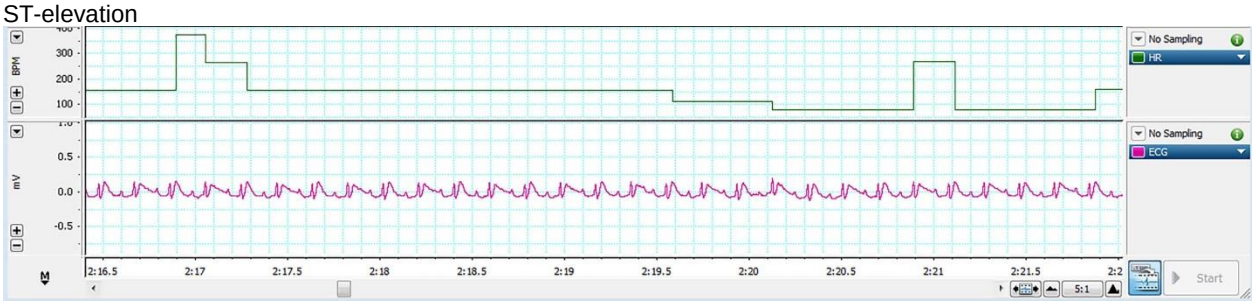
Nonsustained ventricular tachycardia

Complete heart block

Block



ST-elevation



Mobitz type I

Mobitz type II

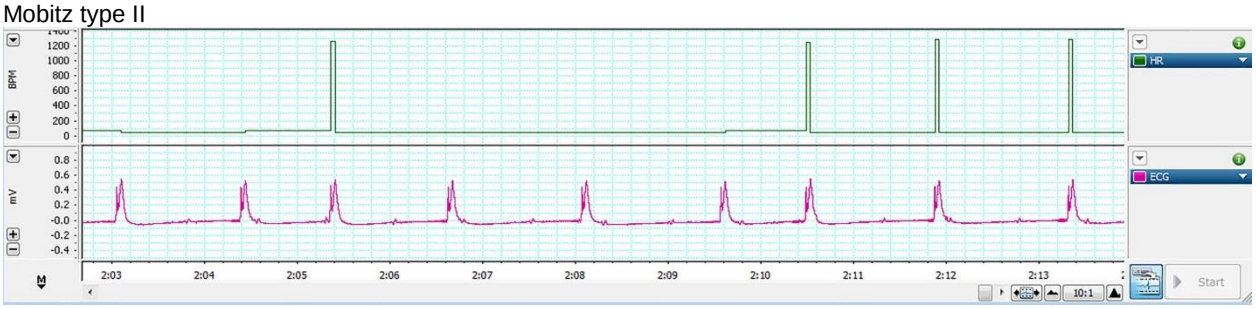


Figure 5: Samples as representatives of lead II electrocardiography were recorded from the rats in the different groups. A, Control; B, Aminophylline for 3 min

Table 1: Arrhythmias in the different groups

Group	Complete Heart Block	Premature Ventricular Beats	Mobitz I	Mobitz II	AV Block I	Complete Heart Block
Aminophylline (3 min)	+	+	+	+	+	+
Aminophylline (20 min)	-	-	-	-	-	-
Normal saline (3 min)	-	-	-	-	-	-
Normal saline (20 min)	-	-	-	-	-	-

DISCUSSION

In this study, neonate rats receiving aminophylline (5 mg/kg) for 3 minutes showed a significant decrease in their heart rates, while their PR interval significantly increased. Additionally, cardiac arrhythmias, comprised of premature ventricular beats, AV blocks (Mobitz I and Mobitz II), complete heart blocks, nonsustained and sustained ventricular tachycardias, and AV dissociations, were observed.

Aminophylline, caffeine, theophylline, and theobromine are known as methylxanthines. [19]

Due to their low solubility, they can combine with a variety of salts and be used for several therapeutic purposes. [21] Methylxanthines are recognized as major pharmacologic agents used in the treatment of neonatal apnea. [22] Aminophylline, as an ethylenediamine salt of theophylline, is commonly used for the treatment of premature neonates with apnea in the NICU and is believed to stimulate the surfactant section and improve respiratory function. [22] When the serum level of theophylline reaches from 10 to 20 mg/L in the blood circulation, it has been documented to be more effective. [19] Aminophylline toxicity, which is seen in levels greater than 20 mg/L, has been reported to have symptoms such as nausea, anorexia, vomiting, tachycardia, stupor, and irritability. [19] Each 1 mg/kg of theophylline administration can lead to a 2-mg/L increase in the serum theophylline. [19] Theophylline toxicity can occur in levels greater than 20 mg/L. [19] The half-time of theophylline reported in humans ranges from 30 to 45 minutes in the plasma. [19] Aminophylline acts as a myocardial and skeletal muscle stimulant, but its effects on the smooth muscles of bronchioles and blood vessels are known to be relaxing. [19] Several aminophylline mechanisms of action, including nonselective phosphodiesterase inhibitors and adenosine receptor antagonists, have been proposed. [23, 24] Aminophylline can also provoke endogenous catecholamine release. [17] Experimental investigations have reported that the positive chronotropic effects of aminophylline are due to catecholamine release. [25] The sympathetic nervous system produces a number of important transduction pathways, one of which is the β-adrenergic pathway, which is activated by catecholamine-binding G-protein–coupled β-adrenergic receptors. The activation of the β-adrenergic receptor can lead to an increase in the intracellular concentration of the second messenger of cyclic 3'5'-adenosine monophosphate (cAMP), and an increase in cAMP triggers the activation of protein kinase. The activation of protein kinase can, in turn, lead to the phosphorylation of a number of proteins that affect electrical activity and contractility. [26] A-kinase anchoring proteins are recognized as the main component of the β-adrenergic/cAMP/protein kinase pathway. One of the lethal arrhythmias is long QT-syndrome, A-kinase anchoring protein mutation, which results in long QT-syndrome. [27]

In the present study, QTc prolongation was observed for neonate rats given aminophylline for 3 minutes. This was probably due to one of the aminophylline mechanisms of action that activates the sympathetic nervous system, leading to the phosphorylation of a number of proteins such as A-kinase anchoring proteins.

In a study by Greenberg et al, [28] aminophylline administration increased the serum theophylline level up to 36 mg/L and produced premature ventricular contraction bigeminy. Ashvin et al [29] reported that in patients with chronic obstructive pulmonary disease who orally received aminophylline, ventricular ectopic beats and heart rates increased. These findings were similar to the findings of the present study.

Some cardiovascular effects of aminophylline are produced by phosphodiesterase inhibition. For instance, the positive chronotropic and inotropic effects in the myocardium are related to the increase in cAMP. [30] A second messenger in the cardiovascular system by hormone stimulation, cAMP results in positive chronotropic and inotropic effects. [31] It has been documented that positive chronotropic effects are related to the increase in cAMP. [31]

Morka et al [32] demonstrated that meconium-induced acute lung injury in adult rabbits during aminophylline administration as a nonselective phosphodiesterase inhibitor increased the mean arterial pressure, heart rate, and heart rate variability. Reportedly aminophylline administration in adults led to

tachycardia, hypertension, and the production of extrasystoles. [33] In an experimental model of right heart failure, it was found that aminophylline administration led to tachycardia and reduced stroke volume; nonetheless, although it decreased the afterload of the right heart, it could not improve cardiac function perfectly. [34]

Acute theophylline toxicity in adults results in atrial flutters, atrial fibrillations, and supraventricular tachycardias. [33] Caffeine is a methylxanthine, and different types of dysrhythmias including supraventricular tachycardias, premature ventricular beats, ventricular tachycardias, and ventricular fibrillations are caused due to caffeine toxicity. [35] Theophylline clearance decreases in newborns and in some diseases such as acute lung edema. [32]

As was mentioned above, one of the aminophylline mechanisms of action is the adenosine receptor antagonist. Recently, the A1-selective agonist has been applied for treating paroxysmal supraventricular tachycardias and atrial fibrillations, as well as for controlling the heart rate. [24] With regard to the effects of adenosine on the control of the heart rate, aminophylline is expected to induce the heart rate. Nevertheless, in this study, the fast injection of aminophylline was likely to lead to the toxicity levels and cause a significant decrease in the heart rate and premature ventricular beats, leading to AV blocks.

In previous research, because of the release of catecholamines, positive chronotropic effects were seen. In the present study, however, a significant reduction in the heart rate occurred, which was possibly related to the gender, age, or race of the animals used. Thus, further investigations into the mechanisms of function in neonate rats need to be conducted in the future.

Although previous studies have documented that the administration of aminophylline leads to an increase in the heart rate, this study showed that the administration of aminophylline caused bradyarrhythmias. We believe that the fast injection of aminophylline is likely to lead to its toxicity level and cause a significant decrease in the heart rate and premature ventricular beats, causing AV blocks.

CONCLUSIONS

As aminophylline administration is common in the NICU, we suggest that the intravenous injection of aminophylline be performed gently or over a period of 20 minutes.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical Considerations

The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and was approved by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. IR.AJUMS.REC.1395.120).

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REFERENCES

1. Turner CJ, Wren C. The epidemiology of arrhythmia in infants: a population-based study. J Paediatr Child Health. 2013;49(4):278-81. doi: 10.1111/jpc.12155.
2. Jaeggi E, Ohman A. Fetal and Neonatal Arrhythmias. Clin Perinatol. 2016;43(1):99-112. doi: 10.1016/j.clp.2015.11.007.
3. Baruteau AE, Perry JC, Sanatani S, Horie M, Dubin AM, at al. Evaluation and management of bradycardia in neonates and children. Eur J Pediatr. 2016; 175(2):151-61. doi: 10.1007/s00431-015-2689-z.
4. Goodacre S, McLeod K. ABC of clinical electrocardiography: Paediatric electrocardiography. Bmj. 2002; 324(7350):1382-5. PMID: 12052812.
5. Badrawi N, Hegazy RA, Tokovic E, Lotfy W, Mahmoud F, Aly H, at al. Arrhythmia in the neonatal intensive care unit. Pediatr Cardiol. 2009;30(3):325-30. doi: 10.1007/s00246-008-9355-4.
6. Kundak AA, Dilli D, Karagol B, Karadag N, Zenciroglu A, Okumus N, Dogan V, Uzunalic N, at al. Non benign neonatal arrhythmias observed in a tertiary neonatal intensive care unit. Indian J Pediatr. 2013;80 (7):555-9. doi: 10.1007/s12098-012-0852-3.
7. Hoffman TM, Wernovsky G, Wieand TS, Cohen MI, Jennings AC, Vetter VL, Godinez RI, Gaynor JW, Spray TL, Rhodes LA, at al. The incidence of arrhythmias in a pediatric cardiac intensive care unit. Pediatr Cardiol. 2002; 23 (6):598-604. doi: 10.1007/s00246-001-0079-y.
8. Bar-Cohen Y, Silka MJ. Sudden cardiac death in pediatrics. Curr Opin Pediatr. 2008; 20(5):517-21. doi: 10.1097/MOP.0b013e32830c9037.
9. Jhang WK, Lee SC, Seo DM, Park JJ. Mechanical circulatory support to control medically intractable arrhythmias in pediatric patients after cardiac surgery. Korean Circ J. 2010;40(9):471-4. doi: 10.4070/kcj.2010.40.9.471.
10. Barnes BJ, Hollands JM. Drug-induced arrhythmias. Crit Care Med. 2010; 38(6):S188-S97. doi: 10.1097/CCM.0b013e3181de112a.
11. Pramanik AK, Rangaswamy N, Gates T. Neonatal respiratory distress: a practical approach to its diagnosis and management. Pediatr Clinics North America. 2015;62(2):453-69. doi: 10.1016/j.pcl.2014.11.008.
12. Reuter S, Moser C, Baack M. Respiratory distress in the newborn. Pediatr Rev.2014;35(10):417-28. doi: 10.1542/pir.35-10-417.
13. Henderson-Smart DJ, De Paoli AG. Prophylactic methylxanthine for prevention of apnoea in preterm infants. Cochrane Database Syst Rev.2010; 8(12): CD000432. doi: 10.1002/14651858.CD000432.pub2.
14. Rodriguez RJ. Management of respiratory distress syndrome: an update. Respir Care. 2003; 48(3): 279-87. PMID: 12667277.
15. Armanian AM, Badiee Z, Afghari R, Salehimehr N, Hassanzade A, Sheikhzadeh S, Tehrani MS, Rezvan G. Prophylactic aminophylline for prevention of apnea at higher-risk preterm neonates. Iran Red Crescent Med J. 2014; 16(8),e12559. doi: 10.5812/ircmj.12559.
16. Tilley SL. Methylxanthines in asthma. Handb Exp Pharmacol. 2011;200:439-56. doi: 10.1007/978-3-642-13443-2_17.
17. Grover S, Jindal A, Bansal A, Singhi SC, at al. Acute bronchial asthma. Indian J Pediatr. 2011;78(11):1388-95. doi: 10.1007/s12098-011-0524-8.
18. Henderson-Smart DJ, Steer PA. Caffeine versus theophylline for apnea in preterm infants. Cochrane Database Syst Rev. 2010;1: 1-19:CD000273. doi: 10.1002/14651858.CD000273.pub2.
19. Riksen NP, Smits P, Rongen GA. The cardiovascular effects of methylxanthines. Methylxanthines: Springer; 2011. 200, p. 413-37. doi: 10.1007/978-3-642-13443-2_16.
20. D'Avila RS, Piva JP, Marostica PJ, Amantea SL. Early administration of two intravenous bolus of aminophylline added to the standard treatment of children with acute asthma.

Respir Med. 2008;102(1):156-61. doi: 10.1016/j.rmed.2007.07.030

21. Monks TJ, Smith RL, Caldwell J. A metabolic and pharmacokinetic comparison of theophylline and aminophylline (theophylline ethylenediamine). J Pharm Pharmacol. 1981; 33:93-7. PMID: 6111606.

22. Neame M, Aragon O, Fernandes RM, Sinha I. Salbutamol or aminophylline for acute severe asthma: how to choose which one, when and why? Archives of Disease in Childhood-Education and Practice. 2015;100(4):215-22. doi: 10.1136/archdischild-2014-306186.

23. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev. 2006; 58(3):488-520. doi: 10.1124/pr.58.3.5.

24. Muller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs.Biochim Biophys Acta.2011;1808(5):1290-308.doi: 10.1016/j.bbamem.2010.12.017.

25. Chiba S, Hashimoto K. Effect of aminophylline on the SA node of the dog heart in situ. Tohoku J Exp Med.1973; 109(2):203-4. PMID: 4722724.

26. 26. Xiang Y, Kobilka BK. Myocyte adrenoceptor signaling pathways. Science. 2003; 300 (5625):1530-2. doi: 10.1126/science.1079206.

27. Chen L, Marquardt ML, Tester DJ, Sampson KJ, Ackerman MJ, Kass RS, at al. Mutation of an A-kinase-anchoring protein causes long-QT syndrome. Proc Natl Acad Sci U S a 2007; 104(52):20990-5. doi: 10.1073/pnas.0710527105.

28. Greenberg, Harry B. Antin, Sidney H. Conduction in Atrial Flutter after Intravenous Injection of Aminophylline. J Electrocardiol.1972; 5(4):391-393. PMID: 4651658.

29. Patel AK, Skatrud JB, Thomsen JH. Cardiac Arrhythmias Due to Oral Aminophylline in Patients with Chronic Obstructive Pulmonary Disease. Chest. 1981; 80(6): 661-5. PMID: 7307587.

30. Hirota K, Yoshioka H, Kabara S, Kudo T, Ishihara H, Matsuki A, at al. A comparison of the relaxant effects of olprinone and aminophylline on methacholine-induced bronchoconstriction in dogs. Anesth Analg. 2001; 93(1):230-3. PMID: 11429372.

31. Miller CL, Yan C. Targeting cyclic nucleotide phosphodiesterase in the heart: therapeutic implications. J Cardiovasc Transl Res. 2010;3(5):507-15. doi: 10.1007/s12265-010-9203-9.

32. D. Mokra IT, H. Pistekova, Z. Visnovcova, J. Mokry, A. Drgova, M. Repcakova, A. Calkovska, at al. Short-term cardiovascular effects of selective phosphodiesterase 3 inhibitor olprinone versus non-selective phosphodiesterase inhibitor aminophylline in a meconium-induced acute lung injury. J Physiol Pharmacol. 2013;64(6):751-9. PMID: 24388890.

33. Aggelopoulou E, Tzortzis S, Tsiourantani F, Agrios I, Lazaridis K. Atrial Fibrillation and Shock: Unmasking Theophylline Toxicity. Med Princ Pract. 2018;27(4):387-91. doi: 10.1159/000490145.