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Review

Caffeine: cardiorespiratory effects and tissue protection in animal models

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Abstract: The aim of this review is to analyze the cardiorespiratory and tissue-protective effects of caffeine in animal models. Peer-reviewed literature published between 1975 and 2021 was retrieved from CAB Abstracts, PubMed, ISI Web of Knowledge, and Scopus. Extracted data were analyzed to address the mechanism of action of caffeine on cardiorespiratory parameters (heart rate and rhythm), vasopressor effects, and some indices of respiratory function; we close this review by discussing the current debate on the research carried out on the effects of caffeine on tissue protection. Adenosine acts through specific receptors and is a negative inotropic and chronotropic agent. Blockage of its cardiac receptors can cause tachycardia (with arrhythmogenic potential) due to the intense activity of β1 receptors. In terms of tissue protection, caffeine inhibits hyperoxia-induced pulmonary inflammation by decreasing proinflammatory cytokine expression in animal models. The protection that caffeine provides to tissues is not limited to the CNS, as studies have demonstrated that it generates attenuation of inflammatory effects in pulmonary tissue. It inhibits the effects of some pro-inflammatory cytokines and prevents functional and structural changes.
Key words: animal model, caffeine, cardiorespiratory effect, tissue protection

Introduction

Caffeine has been part of human diets for thousands of years due to its stimulatory effect and impact on behavior. In 1820, Friedlieb Ferdinand Runge discovered caffeine's chemical structure and relation to the metabolism of nucleic acid, especially purines [1]. Caffeine is a trimethylated xanthine with a molecular structure

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similar to that of adenosine. It acts as a nonspecific inhibitor of two of the four known adenosine receptors, especially A_1 and A_{2A}, which exist in many areas of the brain, lungs, and cardiovascular system [2]. Caffeine is one of the five medications most often prescribed in neonatology [3, 4]. A clinical trial with 1,000 neonates in a caffeine group and 1,000 neonates in a placebo group [5] found reduced bronchopulmonary dysplasia (BPD) and a decrease in the need for medical or surgical management of patent ductus arteriosus (PDA) [5–7]. Another study showed a reduction in the use of vasopressors [8]. Caffeine's pharmacokinetics and proven effects in neonates are shown in Fig. 1.

A study by Alur *et al.* [20] monitored therapeutic levels in 198 premature neonates (<29 weeks of gestation). Those with caffeine levels >14.5 μg/ml had a lower incidence of BPD, fewer interventions for PDA, less ventilator use, shorter hospital stays, and a decreased need for diuretics and oxygen at home.

In the cardiovascular system, caffeine increases ionic Ca^{2+} by inhibiting cyclic adenosine 3',5'-monophosphate (cAMP) [21, 22] and acting as an agonist of the ryanodine receptors linked to those channels [23]. Studies of the respiratory tract show that it affects the flow of intracellular Ca^{2+}, improving the activity of the respiratory muscles [24] and sensitizing the central and peripheral chemoreceptors responsible for controlling respiration [25]. These properties constitute one reason for caffeine being recognized as having a significant therapeutic value in enhancing infants' ventilatory status [26]. In terms of animal research, most work has focused on rodents [27] or non-human primates [28], and few studies have examined its effects on pigs [29–31]. In light of this, the main objective of this review is to analyze the cardiorespiratory and tissue-protective effects of caffeine in animal models.

Caffeine's Action Mechanism Associated with Cardiorespiratory Effects

Caffeine exerts a stimulatory effect on the cardiorespiratory system [32, 33], as its action mechanism includes non-selective antagonism of the A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors in the CNS [34]. Specifically, acting as an antagonist of the A_1 or A_{2B} receptors allows caffeine to activate the inhibitory G protein [21], which acts to inhibit the activity of the kinase A protein enzyme and impede phosphorylation of the Ca^{2+} channels, thus reducing the intracellular flow of this metabolite [22].

In humans, it has been identified that adenosine A_1 receptors are expressed principally in the brain, spinal cord, heart, and autonomic nerve fibers and that A_{2A} receptors are located in the brain, heart, lung, and spleen; furthermore, the main distribution of the A_{2B} receptor, is in the colon and bladder, and the A_3 receptor is expressed in the lung, liver, brain, testicles, and heart [35].

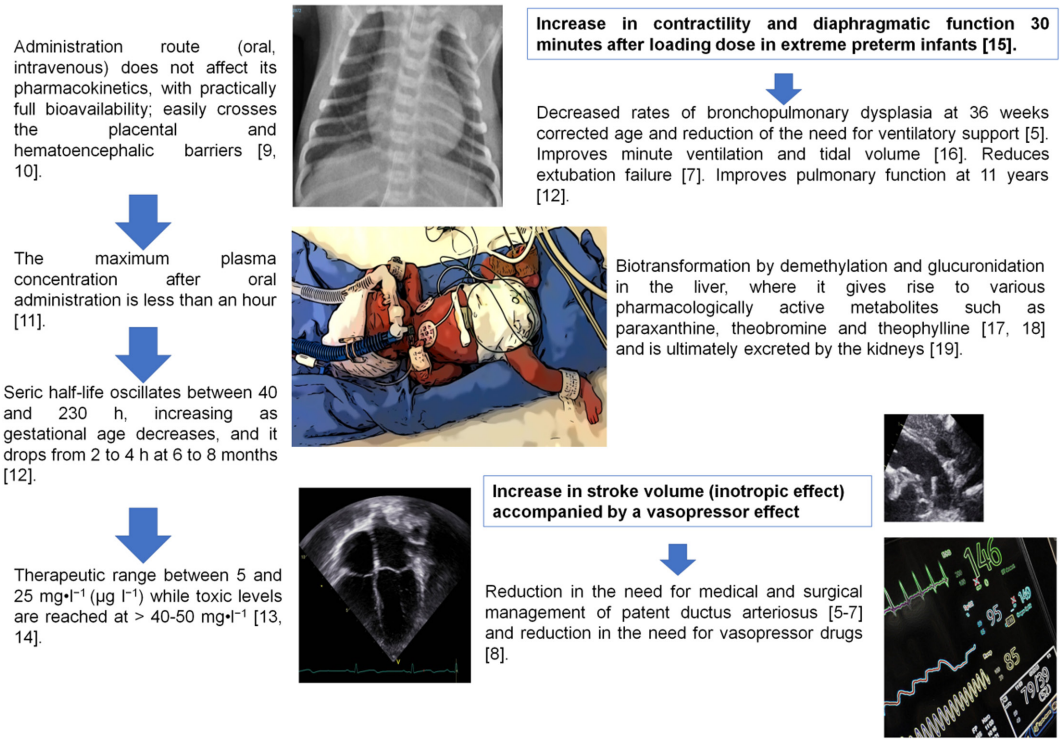

Fig. 1. Pharmacokinetics of caffeine and proven clinical effects in neonates [5–19].

However, there is a difference in the expression of the receptors in the case of animals. For example, it has been identified that rats have a high distribution of A_1 receptor in the CNS, mainly in the cerebral cortex, hippocampus, cerebellum, thalamus, brain stem, and spinal cord, with expression also in the testicle, white adipose tissue, stomach, spleen, adrenal, eye, and bladder [36, 37].

In the case of the A_{2A} receptors, high concentrations are expressed in the spleen, thymus, leukocytes, blood platelets, and CNS, and although their concentrations are low, they are also present in the heart, lung, and blood vessels [38, 39]. Regarding the A_{2B} receptor, it has been found that rats have low levels in all brain regions, but Northern blot revealed that mice and rats have high levels in the cecum, colon, and bladder, in addition to low levels in the spinal cord, lungs, and vas deferens [36–40].

The A_3 receptor is widely distributed in most animals, with pronounced differences between species [39]. It has been detected in the testicle, lung, kidney, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, aorta, colon, and eye in rats, sheep, and humans; however, the presence of this receptor has not been detected in skin and skeletal muscle [34, 35, 41–43].

The mechanism through which caffeine generates physiological effects in the circulatory and respiratory tracts is identical to that of the CNS. Caffeine molecules exert agonist action on ryanodine receptors in the endoplasmic reticulum of cells in the cardiac skeletal muscle associated with Ca^{2+} channels [23, 44]. Caffeine induces a greater sympathetic response that impacts muscular activity [45, 46]. Kraaijenga *et al.* [24] demonstrated that the release of epinephrine due to an increase in Ca^{2+} concentrations in the sarcoplasm improved the contractibility of the diaphragm in full-term newborns.

Regarding the pharmacokinetic parameters of caffeine, the absorption, bioavailability, and excretion processes are generally similar among humans, dogs, rabbits, rats, and mice; however, there is a difference in metabolism among species. For example, the enzyme CYP1A2 is present only in the liver in humans and is responsible for 90% of the plasma clearance of caffeine [43]. In the case of rats, however, this enzyme is responsible for 40% of the plasma clearance of caffeine [47].

Another difference observed between species is the difference in the half-life of caffeine, as it has been identified that it is 80 ±_23 h in humans [47], whereas it is 47.5 ± 5.35 h in dogs [48]. It has also been emphasized that caffeine's half-life increases with age, for example, in dogs, with the half-life in young animals being 41 h longer compared with that in adult animals 6.66 ± 0.85 [48]. This is because the hepatic enzymatic system is immature in puppies causing caffeine elimination to take more time, and it has been identified that the plasmatic half-life in neonates is between 65 to 103 h, decreasing to 14.4 h in infants and subsequently to 3 to 6 h in adults [47, 48].

On the other hand, a difference in the metabolism of caffeine has not been observed between males and females in humans [49], which is significantly different in animal models; in pregnant rats and rabbits, the half-life of caffeine is longer compared with that in nonpregnant females or males, and it has even suggested that caffeine elimination is decreased at the end of gestation [50].

Effects on Heart Rate and Rhythm

Studies with animal models have led to debate regarding whether caffeine produces the same effects on heart rate as those observed in humans (Fig. 2).

Some meta-analyses mention that consuming caffeine (400 mg) increases the frequency of the function of the cardiac conduction system [57–59], an effect also reported in Wistar rats, where observations included an early increase in the heart rate accompanied by higher blood pressure [58].

Caffeine induces a dose-dependent increase in heart rate via an interaction that induces the greater activity of the cardiac fibers, which can trigger ventricular-type tachycardia or modify the auricular rhythm. This dose-dependent effect helps us understand studies of the same species, though Sprague Dawley rats that received a 16-mg/kg intravenous dose of caffeine did not show any observable changes in cardiac rhythm [60]. No increase in heart rate was noted. In other cases, observations did

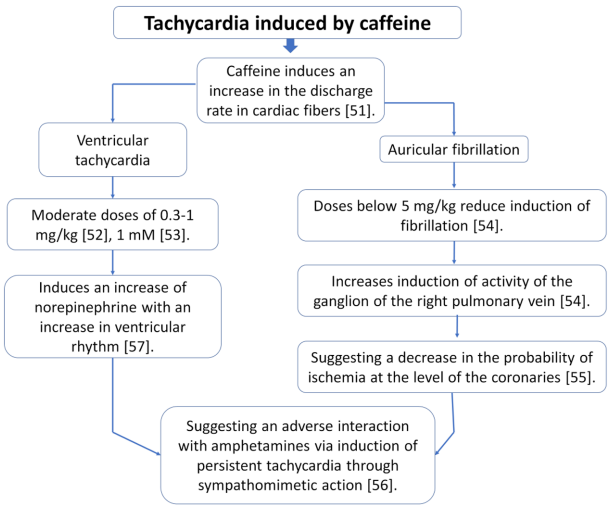

Fig. 2. Tachycardia induced by caffeine. There is a dose-dependent relationship between the increase in frequency and caffeine. This interaction induces greater activity of the cardiac fibers, which can generate ventricular-type tachycardias or modifications of the auricular rhythm [51-62].

show a risk of ventricular-type arrythmias such as ventricular tachycardia and first-degree heart block [58–60].

Caffeine's pro-arrhythmia properties have been amply identified in humans. Mattioli [54], for example, reported a positive association between chronic caffeine consumption and an increased risk of developing atrial fibrillation (AF). The tendency in animals is the same, as a 35-g dose in dogs increased the risk of arrhythmias [55, 63]. Mehta *et al.* [53] demonstrated that administering caffeine to dogs at doses of 1, 2.5, and 5 mg/kg increased the risk of ventricular tachycardia, AF, and ventricular premature complexes. The question has been raised as to whether these pro-arrhythmia effects of caffeine appear in canine species. Rashid *et al.* [64] found that caffeine reduced the possibility of AF presenting in dogs treated with doses of 1, 3, and 5 mg/kg and that increasing the dose of caffeine reduced the risk of AF.

Caffeine overdose can lead to some health problems, such as the increased risk of seizures, the development of arrhythmias, and decreased response to hypoxia. This situation has been observed in dogs and rats, with overdose increasing the predisposition to develop neurotoxic effects. This mechanism is associated with increased glutamate exocytosis, which generates greater neuronal activity [4].

On the other hand, the lethal dose of caffeine in rats has been estimated to be 150–265 mg/kg similar to that in dogs and rhesus monkeys [56, 65]. Although effects such as nervousness, fever, irritability, headache, and sleep disorders have been reported with a dose of 400 mg, they are of relatively short duration and tolerable in humans and monkeys [65]. In other animal models, such as dogs, it has been reported that the toxic dose could be 120–200 mg/kg and that such a dose has effects on the cardiovascular, pulmonary, neurological, gastrointestinal, and metabolic systems due to the inhibition of cyclic nucleotide phosphodiesterase. This mechanism produces positive inotropic, chronotropic, and dromotropic effects on the heart, renal vasodilation, cerebral vasoconstriction, smooth muscle relaxation, and stimulation of gastric secretion [65, 66].

In addition to altering the flow of Ca^{2+}, caffeine modifies the activity of the adenosine receptors [67, 68]. In a study of gestating rats, Iglesias *et al.* [69] observed that chronic caffeine consumption altered the amounts of adenosine receptors in the cardiac myocytes of both the fetus and the mother. This could represent a risk in response to apnea. In this regard, Buscariollo *et al.* [70] evaluated whole murine embryos and isolated hearts *in vitro*. They identified that caffeine eliminated hypoxia-mediated bradycardia by reducing the heart rate and preventing the agonist effect of the A_1 and A_2 receptors. This phenomenon suggests the following association: alteration of the number of receptors derived from the antagonism of the adenosine receptors allows caffeine to induce changes in cardiac function and growth and to modify the methylation of deoxyribonucleic acid, thus aggravating the level of the cardiac system's response to apnea in neonates [71]. These findings led to the notion that caffeine must be used with great caution or avoided completely during gestation.

There are no reports of arrhythmias as an adverse effect in published assays with human neonates. Although increases in heart rate and arterial pressure have been demonstrated [72], the values recorded remained within normal limits (i.e., under the 95th percentile for gestational age). A small study (n=21) showed that caffeine has no effect on the heart rate variability [73] but pointed out that there is a lack of evidence for arrhythmogenic potential in human neonates.

Vasopressor Effects

There is evidence of an association between caffeine consumption and high blood pressure [58, 74–77]. This effect is related to caffeine's antagonistic action on adenosine receptors in the brain, fostering greater secretion of renin [78], and increasing dopaminergic neurotransmitters [79]. Concerning blood pressure, results to date are contradictory, as some authors have found that blood pressure after 30, 60, and 90 min was not affected in rats that received distinct amounts of coffee beans (Arab, Turkish, and American) at oral doses of 250 or 300 mg/kg. Their findings demonstrated a decrease in blood pressure readings [80].

Corsetti *et al.* [81] observed that when administered intravenously at a dose of 16 mg/kg, caffeine increased the mean blood pressure in 20% of male rats but that the higher readings returned to basal values at 30 min post-administration. This vasopressor effect is useful in anesthetized animals. White and Nguyen [82] evaluated the activity of the A_1 and A_2 adenosine receptors in Hooded Wistar rats anesthetized with sodium pentobarbital. They observed that treatment with caffeine at 2% (2 mg/ml) 14 days prior to an experimental period produced a significant increase in blood pressure (147 ± 5 compared with 161 ± 3 mm Hg), leading them to suggest that caffeine could help reverse the vasodilator effects of barbiturates.

Another key study area is the relationship between increased blood pressure due to chronic caffeine consumption and aggravated damage to tissues with a high degree of vascular demand, such as the kidney. Tanner *et al.* [74] evaluated Sprague Dawley rat littermates at 6 months of age. They were given caffeine at 0.1% for

9 weeks. Findings showed that chronic administration of caffeine significantly affected the rate of glomerular filtration due to a prolonged increase in blood pressure that degenerated into the development of renal cysts. However, it was impossible to associate vasopressor effects with angiotensin II, so they concluded that caffeine promotes kidney damage associated with hypertension.

This idea found support in the work by Tofovic *et al.* [75], who found that the renal damage caused by caffeine was not blocked by an antioxidant. They evaluated 37 eleven-week-old obese, diabetic male ZSF1 rats by administering a dose of caffeine at 0.1% with tempol (1 mmol/l) for 9 weeks. They did not observe any effect on blood pressure but did find evidence of an increase in vascular renal resistance and heart rate. In addition, they used immunohistochemical analysis to demonstrate that caffeine induced greater inflammatory renal damage and glomerular fibrosis. Their study concluded that caffeine increases proteinuria and the proliferative mechanisms of kidney damage via the adenosine receptors. Their study and Tanner *et al.* [74] above reveal a negative impact associated primarily with hypertension and secondarily with cell damage derived from renal hypoperfusion.

In humans, the cardiovascular effects of caffeine are characterized by an increase in stroke volume (inotropic effect) accompanied by a vasopressor effect with a reduced need for medical or surgical treatment of PDA and need of vasoactive drugs. Clyman *et al.* [83] studied the ductus arteriosus in 24 preterm fetal lambs *in vitro* to determine the direct effects of caffeine on isometric tension. Caffeine (0.003–0.3 mM) had no direct effect on ductus arteriosus tension, nor did it affect the contractile response of the ductus arteriosus to increasing oxygen concentrations. Their results, however, did not agree with those of an *in vivo* study [5], since their *in vitro* study [83] failed to consider the shear stress that occurs *in vivo* hence, it is possible that caffeine interacts with *shear*-related signaling and acts at some site distant from the ductus, altering the production of circulating substances that might affect its contractility.

Effects on the Respiratory Apparatus

Studies show that, in contrast to its effects on the cardiovascular system, caffeine has a distinct mechanism of action in the respiratory tract. There are four stages in this biochemical mechanism: (i) mobilization of Ca^{2+} at the intracellular level, (ii) inhibition of phosphodiesterases, (iii) modulation of $GABA_A$ receptors, and (iv) antagonism of the A_3 adenosine receptors [14]. This mechanism is responsible for modifying the lung's response to a deficiency in oxygenation or sensitization of the chemoreceptors to O_2 and CO_2 molecules [7]. In a pilot study with newborn mandrills, Yoder *et al.* [84] demonstrated that early treatment with caffeine was associated with enhanced pulmonary function better lung function during the first 24 h of life. In another context, some authors have detected that caffeine can play a key role in preventing and treating episodes of neonatal apnea by stimulating the CNS [85, 86]. However, other work has questioned whether this phenomenon is attributable exclusively to caffeine or one of its metabolites. Skouroliakou *et al.* [87] observed that a combination of standardized doses of caffeine and theophylline administered to neonates at less than 33 weeks of gestation significantly reduced apnea events, whereas administering caffeine alone controlled apnea in at-risk newborns. This demonstrated that caffeine only aids in counteracting apnea and that derivatives of its metabolism help control this event.

Caffeine induces a greater sympathetic response by affecting muscular activity [45]. Regarding this, Kraaijenga *et al.* [24] determined that caffeine affects the activity of the diaphragm and on the tidal volume of newborns. They utilized two techniques: electromyography to measure diaphragmatic activity and inductive respiratory plethysmography to determine changes in tidal volume during the 30 min after intravenous administration of caffeine at a dose of 10 mg/kg for 3 h. Their findings showed an increase in diaphragmatic activity and tidal volume of up to 30%, concluding that caffeine fosters sustained increases of both parameters, possibly via the dopaminergic discharge caused by caffeine [88]. Caffeine has more than one mechanism for reducing apnea, including the possible attenuation of the upper laryngeal nerve and increased diaphragmatic activity to improve ventilation.

Tissue Protection and Caffeine at the Cardiorespiratory Level

Caffeine has also been shown to possess a solid anti-inflammatory capacity. Previous studies with animal models confirmed that–especially at high doses–caffeine exerts significant therapeutic effects on traumatic brain injuries [89] and pulmonary lesions induced by oleic acid and demonstrates protective effects against ischemia-reperfusion of the myocardium [90]. As mentioned above, caffeine protect against inflammation (Fig. 3).

Previous studies also identified caffeine in the CNS [93, 94], especially in individuals with age-induced degenerative changes [95].

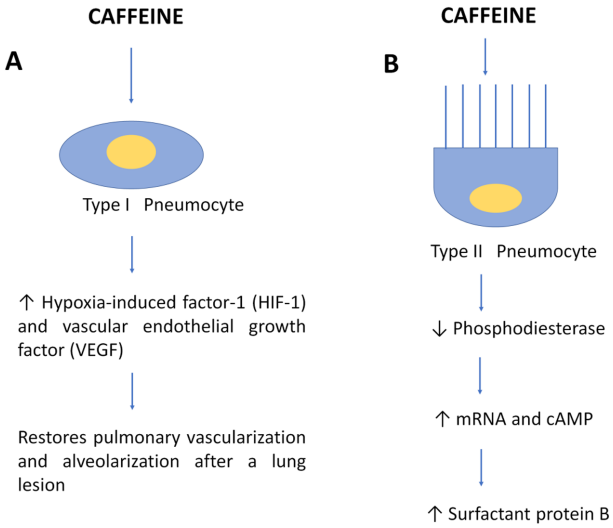

Fig. 3. Effects of caffeine on pneumocytes. A. Stimulation of type I pneumocytes. In addition to hyperoxia, caffeine induces the expression of VEGF and HIF-1, possibly fostering pulmonary revascularization and alveolarization in adult lungs after an acute lesion [91]. B. Stimulation of type II pneumocytes. This suggests an interaction of caffeine (5 mM) with glucocorticoids that increases intracellular levels of cAMP to generate a positive synergetic increase in messenger ribonucleic acid (mRNA) and the B apoprotein with improved homeostasis of the surfactant factor [92].

In the respiratory apparatus, caffeine reduces the expression of pro-inflammatory cytokines like TNF-alpha in the epithelium [96, 97]. However, this aspect requires additional research before any effective therapy can be standardized. On this topic, Nagatomo *et al.* [98] utilized rabbits of the New Zealand breed to study whether caffeine prevents inflammatory, structural, and functional changes. Their work evaluated pulmonary and vascular morphometry and inflammation of the respiratory pathway using a scoring scale. Their observations showed that caffeine induced increases in pulmonary volume, capacity, and elasticity while also reducing scores for acute inflammation.

A study by Aranda *et al.* [99] in newborn rats revealed that the administration of ketorolac or caffeine alone reduced neovascularization of the retina. In contrast they prevented severe induced retinopathy of prematurity (ROP) and improved the maturation of the neural circuit of the retina more efficaciously when combined than when used individually. They also had synergetic or additive effects in the retina and serum growth factors including the hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF). These findings may be useful in developing pharmacotherapies designed to prevent ROP.

The anti-inflammatory effect of caffeine operates through interaction with fibroblasts in the pulmonary tissue, where it reduces levels of pro-inflammatory cells by reducing TNF-alpha levels, as has been demonstrated by combining it with glucocorticoids [97]. Moreover, Fehrholz *et al.* [96] observed that caffeine affects the expression of TGF-beta1 in lung epithelial cells, thus contributing to the protection of this system [96].

Research in this field has not been limited to analyses of tissue-protective effects in the respiratory tract; instead, experimental models have been migrated to the cardiovascular apparatus as well. One recent report shows that administering caffeine reduces the lesion in heart tissue that suffers an infarction, thus preventing zones from being affected by ischemia. Xu-Yong *et al.* [90] evaluated 50 Wistar rats that received caffeine at a dose of 25 mg/kg/day for 4 weeks before the experiment. They found that the lesions caused by infarction in those subjects were significantly decreased compared with those of a control group, with reduced apoptosis of cardiac myocytes. They concluded that the tissue-protective effect was due to suppression of caspase 3 activation, which allowed them to establish that caffeine protects against lesions of this kind.

Ye *et al.* [100] observed that caffeine administration nullified the ischemic effects of the atorvastatin used to reproduce infarction in rats. In addition, it prevented phosphorylation of the kinase B protein (Akt-1), though it did not reduce the size of the affected zone. Studies in this area are limited and have produced conflicting results, so investigation of the protective effects of caffeine in different tissues could well be an essential line of research that needs to be pursued in the development of new therapeutic approaches.

Conclusion

Studies of human neonates have demonstrated that caffeine administration confers beneficial cardiorespiratory effects by increasing tidal volume, stroke volume, and blood pressure. Results regarding its effect on cardiac frequency, however, are contradictory. The results of animal models in this regard are not clear either, likely due to such factors as physiological status, time of consumption, and frequency of administration, which generate unique responses in each individual exposed to caffeine.

The protection that caffeine provides to tissues is not limited to the CNS. Studies have demonstrated that it causes attenuation of inflammatory effects in pulmonary tissue, inhibiting the effects of some pro-inflammatory cytokines and preventing functional and structural changes. Moreover, the same effects have been suggested in studies on heart tissue, which have shown that

caffeine limits the lesions caused by infarction and allows reperfusion. This review suggests the need to conduct additional studies of the effects of caffeine in both animal and human models.

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