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CAFFEINE IMPROVES HEART RATE WITHOUT IMPROVING SEPSIS SURVIVAL

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Abstract

Introduction—Caffeine is consumed on a daily basis for its nervous system stimulant properties and is a global adenosine receptor antagonist. Since adenosine receptors have been found to play a major role in regulating the immune response to a septic insult, we investigated if caffeine consumption prior to a septic insult would alter immunological and physiological responses, as well as survival.

Methods—Two separate experimental designs were employed, both using outbred female ICR mice. In the first experiment mice were administered 20mg/kg of caffeine (equal to 2–3 cups of coffee for a human) or normal saline intraperitoneally at the time of cecal ligation and puncture (CLP). Immunological parameters including cytokines and local cell recruitment measured. In the second experiment caffeine (10mg/kg/hr) was delivered continuously for 24 hours via a subcutaneous infusion pump placed the day prior to CLP and hemodynamic parameters were examined. In both experiments survival was followed for five days.

Results—A single dose of caffeine at the initiation of sepsis did not alter survival. This single dose of caffeine did significantly increase in plasma levels of the chemokine KC six hours after the onset of sepsis compared to septic mice given normal saline. There were no changes in IL-6 or IL-10 levels in the caffeine groups. Peritoneal lavages performed 24 hours post-CLP showed no difference in the levels of IL-6, TNF, KC, MIP-1, IL-10 or the IL-1 receptor antagonist between caffeine and normal saline treated mice. Additionally, the lavages yielded similar numbers of cells (4.1×10^6 vehicle vs. 6.9×10^6 caffeine) and bacterial colony forming units (CFU, 4.1 million CFU vehicle vs. 2.8 million CFU caffeine). In the infusion group, caffeine also did not alter survival. However, caffeine infusion did increase heart rate prior to CLP, and prevented the decline in heart rate after CLP.

Conclusion—Caffeine increased heart rate in mice but does not impact cytokine responses or survival during the acute phase of a polymicrobial sepsis challenge. These data indicate that patients consuming caffeine will not be at risk for increased sepsis mortality.

Introduction

Sepsis continues to be an ever present problem in the medical world with costs rising and a still unacceptable mortality despite new antibiotics or interventions (1, 2). Studies have shown that the number of severe sepsis cases has tripled from a prevalence of 0.5 to 1.5 cases/1000 persons (3). The rate of septic shock has increased more than 6 fold in the United States in the past eleven years (4) although sepsis mortality has recently been stable (5).

One of the problems complicating the delivery of healthcare is the abundance of over the counter substances that people consume on a daily basis, many of which have unknown effects on the immune system. Caffeine is one such substance, consumed daily for its stimulatory effects in the form of coffee, tea, and energy drinks. In the US the average person will consume approximately 3mg/kg/day of caffeine, mostly coming from coffee (6). Caffeine consumption by adults has been estimated to be 106 to 170 mg/day, when considering all sources (7). An updated survey published in 2014 of 37,602 people in the United States demonstrated that the daily caffeine intake was 165 mg/day (8). Caffeine alters the immune system by acting as an antagonist on all four adenosine receptors; A₁, A_{2A}, A_{2B}, and A₃ (9). Adenosine acting through its receptors is able to modulate cytokine and chemokine release and affect cell function (10).

Adenosine is released by tissues such as damaged blood vessels when a hypoxic environment is created. The purpose of this increase in extracellular adenosine is to inhibit the overactive immune cells and reduce bystander damage during the response to damage associated molecular patterns (DAMPs) (11). This effect was shown in a study where a hypoxic environment in the lung resulted in a lesser degree of lung injury when compared with mice who breathed air with higher partial pressures of oxygen (12). This group also showed the protective effect of adenosine communicating through the A_{2A} receptor since mice genetically modified to knock out the receptor had worse lung injury after LPS exposure. Others have examined the role of adenosine receptors in polymicrobial septic challenges and have shown improved survival when specifically antagonizing the A_{2A} or A_{2B} receptors (11, 13).

Caffeine has been shown by one group to promote inflammation and liver injury in a model utilizing concanavalin A (con A). In this study mice were given a dose of caffeine with liver injury or allowed to imbibe caffeine on their own. Both methods resulted in worse liver injury and higher levels of pro-inflammatory cytokines (14). This same group also showed that caffeine at 100mg/kg prevented the liver injury produced by con A but this action was not performed through adenosine receptors. This protective effect in other models of liver injury has been documented through decreased transaminase levels (15). A recent epidemiology study showed that consumption of beverages containing caffeine increased lower urinary tract symptoms typically found in patients with urinary tract infections (16). Coffee consumption has also been linked to other deleterious health outcomes, such as increasing the risk of myocardial infarction (17), although a study in rats demonstrated that caffeine provided protection from ischemia/reperfusion injury (18).

If an important role of adenosine is to serve as a stop signal for the immune response and thus prevent bystander damage, then consumption of caffeine would place the immune system in a pro-inflammatory state by blocking the biological action(s) of adenosine. The current study tested the hypothesis that ingesting caffeine may have deleterious consequences on multiple aspects of the immune system in a polymicrobial sepsis model which may result in decreased survival.

Materials and Methods

Mice

Adult female ICR mice (from Harlan-Sprague Dawley, Inc., Indianapolis, IN) were used. Mice were acclimatized to our housing room for at least 5 days before surgery. Animals were housed in a temperature and humidity controlled room with 12 hours light-12 hours dark diurnal cycle. They were provided food and water *ad libitum* for the entire duration of the experiment. The experiments were approved by Boston University Animal Care and Use Committee.

Caffeine

Caffeine (anhydrous) was purchased from MP pharmaceuticals (Solon, OH).

Sepsis and blood sampling

Bacterial peritonitis was induced in mice using our standard model of cecal ligation and puncture (CLP) using our previous methods (19) based on modifications of the original description of the procedure (20). Anesthesia was induced with 5% isoflurane and maintained with 3% isoflurane. The mouse abdomen was washed with chlorhexidine and a midline incision was made. The cecum was identified, extruded from the abdomen and ligated at 1/3 the distance from the ileocecal valve to the end of the cecum. The cecum was punctured twice with an 18 gauge needle that was inserted from the tip of the cecum towards the ligated portion, a small amount of stool was extruded to ensure the patency of the perforations. The cecum was then returned into the abdomen and the abdominal wall was closed with 4-0 silk sutures. The skin was closed using Vetbond (3M, St. Paul, MN). The mouse was allowed to recover in a clean cage. One mL of Normal Saline containing 0.05mg/kg of buprenorphine and 25mg/kg of imipenem was given via the subcutaneous route immediately post-operatively. Buprenorphine was given for a total of 4 doses spaced 12 hours apart. Imipenem was given for a total of 5 days in Lactated Ringers with 5% dextrose. Blood samples of 20µL in volume were collected at 6 and 24 hours post-CLP via facial vein sampling using a 23 gauge needle. Blood was placed into 180µL of 1X PBS with EDTA, centrifuged for 5 minutes, and the supernatants were stored at -20°C until they were ready to be analyzed.

Experimental design

Mice were divided into two experimental groups. For the first experimental group, mice received a single dose of 20mg/kg of caffeine included in the 1st dose of pain medication and antibiotics which was given subcutaneously. The controls received normal saline in

place of caffeine in a similar manner. In the second experimental group, mice had an osmotic pump placed subcutaneously the day before CLP which gave the mice a constant infusion of caffeine (10mg/kg/hr) for a total of 24 hrs. Normal saline was infused using the osmotic pumps in a separate group of control mice. Since the experiments were designed to evaluate the impact of caffeine on the acute phase of sepsis, mice were followed for survival and vital signs measurements for five days. A group of mice from the single dose caffeine group was sacrificed at 24hrs post-CLP in order to perform peritoneal lavages. The absorption and pharmacokinetics of caffeine have been reported to be similar in animals and humans (21).

Osmotic infusion pump placement

Osmotic infusion pumps were purchased from Alzet, Cupertino, CA (Model 2001D). Mice were induced with 5% isoflurane and anesthesia was maintained with 3% isoflurane. The mouse was laid on its abdomen and the dorsal surface washed with Chlorhexidine. A small incision was made and a pocket created in the subcutaneous tissue. The osmotic pump was inserted into the pocket and the skin closed with Vetbond (3M, St. Paul, MN).

Peritoneal lavages

Mice were given a mixture of ketamine/xylazine intraperitoneally and blood collected from the retro-orbital venous plexus. The abdominal area was washed with chlorhexidine and an incision was made parallel to the initial incision for CLP. The abdominal cavity was irrigated with a total of 10ml of HBSS containing EDTA. The lavage fluid was then processed for cell and bacterial counts and for cytokine analysis. The peritoneal cells collected were then centrifuged, red blood cells were lysed with 3 ml of ACK lysis buffer (Invitrogen) and cells were re-suspended in HBSS and counted using Beckman-Coulter particle counter model ZF (Coulter Electronics, Hialeah, FL). Supernatants were stored at -20°C until they were analyzed.

Measurement of physiologic parameters

Heart rate, respiratory rate, pulse distension, and pulse oximetry were recorded using a mouse collar clip device from Starr Life Science (Allison Park, PA). Readings were taken at baseline, 16 hours after placement of osmotic infusion pump but prior to CLP, and then at 2, 6, and 24 hrs post-CLP.

Statistics

Survival was analyzed by log rank analysis and differences between groups were determined by student's t-test using Graphpad. To determine the impact of both caffeine and survival a two ANOVA was employed and analyzed with the Graphpad software. Graphpad was also used to determine the area under the curve for the receiver operator characteristic. Sample size calculation was performed with the G*Power3 software (22).

Results

Acute caffeine administration does not alter sepsis survival

The first experiment sought to determine if acute exposure to caffeine would alter the mortality of polymicrobial sepsis induced by CLP. Caffeine was given subcutaneously to ensure uniform deliver of the full dose. Caffeine has been shown to have similar pharmacokinetics in humans and mice (21). It should be noted that these experiments were not designed to evaluate adenosine receptors, but rather to determine if a commonly consumed substance alters the inflammatory response. Since the initial immune response to a polymicrobial challenge during CLP develops within the first 6 hrs, caffeine was given immediately after surgery to block the adenosine anti-inflammatory signal (23). Caffeine administered at this time did not affect overall survival nor did it change the time at which the deaths occurred as shown in Figure 1. Since there was no impact on the mortality we sought to determine if caffeine would modulate aspects of the inflammatory response, in order to verify that an immunomodulatory dose had been given.

Caffeine increases chemokine production

Plasma levels of several inflammatory mediators were measured in blood collected six hours after the onset of sepsis, a time when several pro-inflammatory and anti-inflammatory mediators are increased (19, 24). IL-6 has been widely used as a marker of the severity of the inflammatory response in CLP (23, 25). The cytokine response is dramatically different in mice that live compared to those who die and blood was collected without sacrificing the mice. This allowed us to separate the mice into those who lived to day 5 compared to those who died by day 5. We measured cytokines in the peripheral blood and in the first analysis mice were grouped into survival status at day 5 (alive vs dead) and not treatment status (normal saline vs caffeine) As expected, mice who will die during the acute phase of sepsis have higher levels of IL-6, the CXC chemokine KC, and the anti-inflammatory cytokine IL-10 (Figure 2). Even when the mice had been given caffeine, a receiver operator characteristic curve analysis showed that IL-6 levels predicted mortality.

These data were further analyzed to determine if caffeine administration given at the onset of sepsis would alter cytokine production even though there was no impact on survival. Mice were divided into their status at day 5 (alive or dead) and whether they were given normal saline or caffeine. Caffeine administration did not alter the plasma levels of IL-6 or IL-10 (Figure 3). A sample size analysis showed that 71 mice would need to be examined to show a significant difference in IL-6 in the normal saline vs. caffeine mice in the dead group, and 156 mice would be needed to show a difference in IL-10 in the dead group. However, caffeine did cause a significant increase in KC among those mice who survived. Previous reports using a cancer cell line demonstrated that pre-treatment with caffeine would decrease chemokine production (26), but our *in vivo* results did not confirm this finding. Although the *in vivo* studies did not replicate the *in vitro* studies, our results did show that the dose and route of administration of the caffeine did modulate the inflammatory response.

Local (peritoneal) inflammatory response

Cytokines were also measured in the peritoneal fluid 24hrs after CLP to determine if caffeine would alter cytokine levels. Since the mice were sacrificed, we could not separate the mice into alive and dead as was done in figure 3. Additional cytokines could be measured due to the larger sample volume obtained from the peritoneal lavage. The pro-inflammatory cytokines IL-6 and TNF, the chemokines KC and MIP-2, and the cytokine inhibitors IL-10 and IL-1 receptor antagonist (IL-1RA) were measured and none of the peritoneal cytokine levels were different in those mice who received caffeine (Figure 4).

We also evaluated whether caffeine had any effect on cell recruitment or bacterial growth at the nidus of infection. The total number of peritoneal cells and bacterial counts were quantified to see if cell recruitment or bacterial killing had been affected by administration of caffeine. While mice that received caffeine had more cells with the peritoneal cavity, this was not significantly higher. Sample size analysis showed that 57 mice would be needed to demonstrate a difference between the groups. Bacterial counts were found to be comparable between the groups (Figure 5).

Caffeine delivered via infusion – second experimental protocol

Caffeine has a short half life and it was possible that a single dose of caffeine given at the initiation of sepsis would not replicate frequent coffee drinker who develops an inflammatory insult. In order to achieve a sufficient and constant dose we implanted osmotic infusion pumps subcutaneously that would deliver a dose of 10mg/kg per hour for a total of 24hrs. This dose is half of what the mice in the previous experimental set of mice received but it is still considered a pro-inflammatory dose (14). The change in dose was necessary due to the constraint in the size of infusion pump that was able to be implanted in the mice. The pump was placed in the mice 16 hours prior to performing CLP and delivered caffeine up to 6 hours post-CLP. Caffeine administered via an infusion pump at 10mg/kg/hr for 24hrs did not change the survival of CLP when compared to mice that received normal saline via the osmotic pump (Figure 6). While there was a slight decrease in survival with caffeine infusion, a sample size analysis showed that more than 150 mice would need to be studied to show a statistically significant decline. The survival in the saline group appears to be better than saline group in figure 1, but the actual survival was not statistically significant.

Physiological effects of caffeine

Since caffeine did not alter mortality we examined if caffeine, a stimulatory drug, would affect the hemodynamic parameters of the mice prior to CLP. We also examined if caffeine would alter the post-septic physiological response. The purpose of these studies was to demonstrate that administration of caffeine was sufficient to impact the host so we did not attempt to reproduce the immunological parameters in the first study. Physiological parameters were measured prior to pump placement, and 16 hours later immediately before CLP was performed. Mice that received the infusion pump containing caffeine experienced a significant increase in their heart rate of 94 beats per minute (Figure 7A and B). In fact, every mouse that received caffeine increased their heart rate, while the mice that received infusion pumps filled with normal saline did not have a change in their heart rate. After CLP mice that received caffeine through the pump had smaller changes in hemodynamic

parameters than the normal saline mice. In addition to the initial increase in heart rate, caffeine prevented the early drop in blood pressure after CLP. Up to 24 hours after CLP caffeine treated mice still had higher heart rates compared to normal saline mice (Figure 7C).

Discussion

With the consumption of caffeine on a daily basis by people, we sought to understand its effects on the septic response, since coffee drinkers may develop sepsis. In the age of antibiotic resistant bacteria, it is important to elucidate the effect of caffeine on the immune system, especially with its interactions with the adenosine receptors (27, 28). These receptors have been shown to play important roles in anti-tumor research since blocking the A_{2A}R improved tumor rejection in a mouse model (29).

Utilizing a CLP model of polymicrobial sepsis we demonstrated that caffeine at doses typically ingested by normal individuals does not affect the survival during the acute phase of CLP. These results differ from others that have shown that antagonizing individual adenosine receptors, specifically A_{2A}, has been shown to improve survival in CLP (30). This difference could be due the nature of selective versus global antagonism of adenosine receptors. In the peripheral blood it appears that caffeine promotes a slight pro-inflammatory state as higher levels of the chemokine (KC) were present with equivalent levels of anti-inflammatory cytokines. Caffeine levels above what a typical patient would ingest were not utilized since these have been shown to act in pathways other than the adenosine receptors. Our data did show increases in heart rate after caffeine with minimal changes in inflammatory parameters. The ability to influence cardiovascular parameter but not immune parameters may relate to differences in adenosine receptors on different cell types. A limitation of the study is that only female mice were examined.

Our results do not agree with previous published works in which caffeine is mostly labeled as an anti-inflammatory substance at doses that are normally consumed by humans. These effects have been defined by decreased antibody production in murine splenocytes, decreased lymphocyte proliferation, and decreased pro-inflammatory cytokine production (31). Most of the models reviewed use a single type of challenge compared to our model of polymicrobial sepsis. To add to the discrepancies one group administered caffeine to humans after LPS exposure and found no difference in cytokine profiles or measured end organ damage when comparing to placebo (32) while others have shown the deleterious effects of caffeine during ischemia reperfusion, again in humans (33).

Adenosine and caffeine have been both shown to impact the vascular system. Adenosine functions as a vasodilator while co-administration of caffeine can block this effect (34). Our experiments utilizing caffeine resulted in improved hemodynamic parameters when compared to the mice that received only normal saline. These results agree with previous work using rats given caffeine after CLP which showed improved cardiac function when compared to controls. However, this work also showed improved survival following CLP (35). The improved survival with caffeine in this report probably is probably due to the high mortality in the vehicle group.

Caffeine has been shown to be a non-selective adenosine receptor antagonist capable of exerting its effect at concentrations that typical coffee consumers will achieve. Adenosine has been shown to be protective in tissues during inflammatory responses with caffeine blocking this action and maintaining the inflammatory response to a particular stimulus. Data reported in the current manuscript indicate that the typical caffeine intake in a human will have a small affect inflammatory and physiologic parameters, but not survival in sepsis.

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References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med.* 2001; 29(7):1303–10. [PubMed: 11445675]
2. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med.* 2003; 348(16):1546–54. [PubMed: 12700374]
3. Linde-Zwirble WT, Angus DC. Severe sepsis epidemiology: sampling, selection, and society. *Crit Care.* 2004; 8(4):222–6. [PubMed: 15312201]
4. Walkey AJ, Wiener RS, Lindenauer PK. Utilization patterns and outcomes associated with central venous catheter in septic shock: a population-based study. *Crit Care Med.* 2013; 41(6):1450–7. [PubMed: 23507718]
5. Stevenson EK, Rubenstein AR, Radin GT, Wiener RS, Walkey AJ. Two decades of mortality trends among patients with severe sepsis: a comparative meta-analysis*. *Crit Care Med.* 2014; 42(3):625–31. [PubMed: 24201173]
6. Barone JJ, Roberts HR. Caffeine consumption. *Food Chem Toxicol.* 1996; 34(1):119–29. [PubMed: 8603790]
7. Knight CA, Knight I, Mitchell DC, Zepp JE. Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. *Food Chem Toxicol.* 2004; 42(12):1923–30. [PubMed: 15500929]
8. Mitchell DC, Knight CA, Hockenberry J, Teplansky R, Hartman TJ. Beverage caffeine intakes in the U.S. *Food Chem Toxicol.* 2014; 63:136–42. [PubMed: 24189158]
9. Fredholm BB. Astra Award Lecture. Adenosine, adenosine receptors and the actions of caffeine. *Pharmacol Toxicol.* 1995; 76(2):93–101. [PubMed: 7746802]
10. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov.* 2006; 5(3):247–64. [PubMed: 16518376]
11. Belikoff B, Hatfield S, Sitkovsky M, Remick DG. Adenosine negative feedback on A2A adenosine receptors mediates hyporesponsiveness in chronically septic mice. *Shock.* 2011; 35(4):382–7. [PubMed: 21192284]
12. Hatfield S, Belikoff B, Lukashev D, Sitkovsky M, Ohta A. The antihypoxia-adenosinergic pathogenesis as a result of collateral damage by overactive immune cells. *J Leukoc Biol.* 2009; 86(3):545–8. [PubMed: 19564571]
13. Belikoff BG, Hatfield S, Georgiev P, Ohta A, Lukashev D, Buras JA, Remick DG, Sitkovsky M. A2B adenosine receptor blockade enhances macrophage-mediated bacterial phagocytosis and improves polymicrobial sepsis survival in mice. *J Immunol.* 2011; 186(4):2444–53. [PubMed: 21242513]
14. Ohta A, Lukashev D, Jackson EK, Fredholm BB, Sitkovsky M. 1,3,7-trimethylxanthine (caffeine) may exacerbate acute inflammatory liver injury by weakening the physiological immunosuppressive mechanism. *J Immunol.* 2007; 179(11):7431–8. [PubMed: 18025187]

15. He P, Noda Y, Sugiyama K. Suppressive effect of coffee on lipopolysaccharide-induced hepatitis in D-galactosamine-sensitized rats. *Biosci Biotechnol Biochem*. 2001; 65(8):1924–7. [PubMed: 11577746]
16. Maserejian NN, Wager CG, Giovannucci EL, Curto TM, McVary KT, McKinlay JB. Intake of caffeinated, carbonated, or citrus beverage types and development of lower urinary tract symptoms in men and women. *Am J Epidemiol*. 2013; 177(12):1399–410. [PubMed: 23722012]
17. Nawrot TS, Perez L, Kunzli N, Munters E, Nemery B. Public health importance of triggers of myocardial infarction: a comparative risk assessment. *Lancet*. 2011; 377(9767):732–40. [PubMed: 21353301]
18. Li XY, Xu L, Lin GS, Jiang XJ, Wang T, Lu JJ, Zeng B. Protective effect of caffeine administration on myocardial ischemia/reperfusion injury in rats. *Shock*. 2011; 36(3):289–94. [PubMed: 21558980]
19. Osuchowski MF, Craciun F, Weixelbaumer KM, Duffy ER, Remick DG. Sepsis Chronically in MARS: Systemic Cytokine Responses Are Always Mixed Regardless of the Outcome, Magnitude, or Phase of Sepsis. *J Immunol*. 2012; 189(9):4648–56. [PubMed: 23008446]
20. Baker CC, Chaudry IH, Gaines HO, Baue AE. Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. *Surgery*. 1983; 94:331–335. [PubMed: 6879447]
21. Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev*. 1999; 51(1):83–133. [PubMed: 10049999]
22. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007; 39(2): 175–91. [PubMed: 17695343]
23. Remick DG, Bolgos GR, Siddiqui J, Shin J, Nemzek JA. Six at six: interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. *Shock*. 2002; 17(6):463–7. [PubMed: 12069181]
24. Moitra R, Beal DR, Belikoff BG, Remick DG. Presence of preexisting antibodies mediates survival in sepsis. *Shock*. 2012; 37(1):56–62. [PubMed: 21921828]
25. Turnbull IR, Javadi P, Buchman TG, Hotchkiss RS, Karl IE, Coopersmith CM. Antibiotics improve survival in sepsis independent of injury severity but do not change mortality in mice with markedly elevated interleukin 6 levels. *Shock*. 2004; 21(2):121–5. [PubMed: 14752284]
26. Merighi S, Benini A, Mirandola P, Gessi S, Varani K, Simioni C, Leung E, MacLennan S, Baraldi PG, Borea PA. Caffeine inhibits adenosine-induced accumulation of hypoxia-inducible factor-1 α , vascular endothelial growth factor, and interleukin-8 expression in hypoxic human colon cancer cells. *Mol Pharmacol*. 2007; 72(2):395–406. [PubMed: 17488804]
27. Olivares J, Bernardini A, Garcia-Leon G, Corona F, BSM, Martinez JL. The intrinsic resistome of bacterial pathogens. *Front Microbiol*. 2013; 4:103. [PubMed: 23641241]
28. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. *Front Microbiol*. 2010; 1:134. [PubMed: 21687759]
29. Sitkovsky M, Lukashev D, Deaglio S, Dwyer K, Robson SC, Ohta A. Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells. *Br J Pharmacol*. 2008; 153(Suppl 1):S457–64. [PubMed: 18311159]
30. Nemeth ZH, Csoka B, Wilmanski J, Xu D, Lu Q, Ledent C, Deitch EA, Pacher P, Spolarics Z, Hasko G. Adenosine A2A receptor inactivation increases survival in polymicrobial sepsis. *J Immunol*. 2006; 176(9):5616–26. [PubMed: 16622031]
31. Horrigan LA, Kelly JP, Connor TJ. Immunomodulatory effects of caffeine: friend or foe? *Pharmacol Ther*. 2006; 111(3):877–92. [PubMed: 16540173]
32. Ramackers BP, Riksen NP, van den Broek P, Franke B, Peters WH, van der Hoeven JG, Smits P, Pickkers P. Circulating adenosine increases during human experimental endotoxemia but blockade of its receptor does not influence the immune response and subsequent organ injury. *Crit Care*. 2011; 15(1):R3. [PubMed: 21211004]

33. Riksen NP, Zhou Z, Oyen WJ, Jaspers R, Ramakers BP, Brouwer RM, Boerman OC, Steinmetz N, Smits P, Rongen GA. Caffeine prevents protection in two human models of ischemic preconditioning. *J Am Coll Cardiol*. 2006; 48(4):700–7. [PubMed: 16904537]
34. Smits P, Lenders JW, Thien T. Caffeine and theophylline attenuate adenosine-induced vasodilation in humans. *Clin Pharmacol Ther*. 1990; 48(4):410–8. [PubMed: 2225701]
35. Verma R, Huang Z, Deutschman CS, Levy RJ. Caffeine restores myocardial cytochrome oxidase activity and improves cardiac function during sepsis. *Crit Care Med*. 2009; 37(4):1397–402. [PubMed: 19242342]

Figure 1.

Impact of caffeine on sepsis survival. Caffeine (20mg/kg) was given as a one time subcutaneous dose at the initiation of sepsis induced by cecal ligation and puncture. Caffeine has no impact on the survival of a polymicrobial insult. Vehicle n=37, Caffeine n=25.

Figure 2.

Plasma cytokine concentrations 6 hours post CLP. Blood samples were collected 6 hours post-CLP and survival followed until day 5. Mice were grouped based on their survival status on day 5, regardless of whether they received caffeine or normal saline. Mice that die in the early phase of sepsis had significantly higher concentrations of cytokines (p values shown in each graph). Each value is the mean $\pm$ SEM, N= 22–31 mice for alive group and 17–24 for dead group. A receiver operator characteristic curve for IL-6 showed an area under the curve equal to 0.84 demonstrating that even when mice are given caffeine IL-6 serves as a biomarker for mortality.

Figure 3.

Plasma cytokine concentrations obtained 6 hours after CLP. Mice were grouped according to survival status at day 5 and treatment (normal saline or caffeine). A single dose of caffeine (20 mg/kg) given subcutaneously at the time of surgery did not change plasma levels of IL-6 or IL-10 in either the alive or dead groups. However, this single dose did result in a significant increase in KC levels in those mice who survived. None of the other groups had a difference. * = $p < .05$ comparing normal saline vs. caffeine in the Alive group. Each bar is the mean $\pm$ SEM. For IL-6 Alive: N= 18 in the NS group, N=13 in the caffeine group, Dead: N=17 in the NS group, N= 7 in the caffeine group. For KC Alive: N=9 in the NS group, N=13 in the caffeine group, Dead: N=13 in the NS group, N=4 in the caffeine group. For IL-10 Alive: N=16 for the NS group, N=13 for the caffeine group, Dead: N=14 for the NS group, N=4 for the caffeine group.

Figure 4.

Peritoneal cytokines 24hrs post CLP. Mice were sacrificed and the cytokines in the peritoneal lavage fluid measured. The cytokines concentrations were equivalent in the mice given either normal saline or caffeine. Each value is the mean $\pm$ SEM, N = 8 for normal saline and N = 12 for caffeine.

Figure 5.

Peritoneal inflammatory cell count and bacterial counts 24 hours post CLP. Mice were sacrificed and the peritoneum lavaged. Inflammatory cell recruitment and bacterial counts were measured. Peritoneal and bacterial counts were similar in mice that received caffeine or mice that received normal saline. Each value is the mean $\pm$ SEM, cell count group N = 8 normal saline and N = 12 caffeine, bacterial count group N = 5 normal saline and N = 9 caffeine.

Figure 6.

Caffeine infusion does not alter sepsis mortality. Caffeine administered via an infusion pump at 10mg/kg/hr did not change the survival of CLP when compared to mice that received normal saline. The pumps were implanted 16 hours prior to CLP. N = 7 for caffeine and N = 9 for normal saline.

Figure 7.

Caffeine infusion effect on heart rate (HR). The heart rate was measured prior to the subcutaneous placement of the osmotic pump to deliver vehicle or caffeine at a dose of 10 mg/kg/hour. HR was measured again 16 hours later, prior to performing CLP. (A) Infusion of the vehicle (normal saline) did not increase the heart rate. (B) Caffeine significantly increased the heart rate prior to CLP. $*=p<.01$ comparing HR prior to caffeine by paired student's t-test. Each symbol is an individual animal and the line connects the before and after measurements for that animal. (C) Caffeine infusion prevents sepsis induced decline in heart rate (HR). HR was measured at the indicated time points and normalized to the pre-CLP values for each mouse. The presence of caffeine prevented the initial decline in HR at 2 hours post CLP. $*=p<.05$ compared to pre-CLP value, $\#=p<0.5$ compared to time 0 values. Each value is the mean $\pm$ SEM.