

Effects of COX-1 and COX-2 Inhibitors in L- Nitro-L- Arginine Methyl Ester Induced Hypertensive Rats

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Abstract

Administration of L-arginine analogues, such as N-nitro-L-arginine methyl ester (L-NAME) is related with cardiovascular and renal functional alteration. The present experiment was planned to study the possible hemodynamic, renal and liver effects of cyclooxygenase (COX)-1(Aspirin) and COX-2 (Celecoxib) inhibitors in L-NAME induce hypertensive rats. The experimental rats were divided into four groups, each with six animals and the treatments were continued for 2 weeks as the following: Group 1: Control. The rats were given standard rat chow and tap water ad libitum. Group 2: L-NAME. The rats were given standard rat chow and L-NAME (20mg/kg body weight). Group 3: COX-1 inhibitor. The rats were supplied with standard rat chow with aspirin (1000 mg /kg diet) and L-NAME Group 4: COX-2 inhibitor . The rats were supplied with standard rat chow with celecoxib (1000 mg /kg diet) and L-NAME. Results showed that the systolic blood pressure (SBP) was elevated in control rats in comparison with L-NAME group. In the group of receiving COX-1 inhibitor, SBP significantly reduced, while COX-2 inhibitor reduced it but not significantly. Heart rate (H.R) was also changed after COX-2 inhibitor administration ,while COX-1 inhibitor did not change it significantly. COX-1 administration increased serum Na⁺ levels ,while serum K⁺ levels was significantly increased in COX-2 group rats compared with the L-NAME group. Supplementation of L-NAME for 15 days produced a significant increase in serum aspartate transaminase (AST) activity compared with the control group. Statistical analysis revealed that a significant reduction in alanine transaminase (ALT) activity was observed by COX-2 administration compared with the L-NAME. COX-2 inhibitor markedly elevated serum creatinine level compared with the L-NAME group. In conclusions, the results suggested that aspirin rather than celecoxib reduces SBP and in contrast to aspirin, celecoxib alter kidney functions through elevation of serum creatinine level, but it may attenuate liver functions through reduction of elevated serum ALT activity by L-NAME administration.

Keywords: COX , Aspirin , Celecoxib, N-Nitro-L-Arginine Methyl Ester

1. Introduction

Administration of L-arginine analogues, such as N-nitro-L-arginine methyl ester (L-NAME) are related with cardiovascular and renal functional alteration (Kunes *et al.*, 2004). The mechanisms by which L-NAME produce hypertension may be due to vasoconstrictor effect of angiotensin II (Ag II) which in turn removes the normal modulatory influence of nitric oxide (NO). Furthermore, previous researchers (Navarow *et al.*, 1994; Zanchi *et al.*, 1995) found that long-term inhibition of NO synthase may due to increase in plasma epinephrine and norepinephrine, consequently they increase blood pressure (BP). Some of the experimental models showed that hypertension produced by NO synthesis inhibitor is association with increased oxidative stress (Sainz *et al.*, 2005).

The prostaglandins perform a homeostatic role within the normal and compromised kidney (Imig,2000) Two isoforms of COX have been identified: COX1 and COX2. COX1 is constitutively expressed in most tissues examined and is thought to be involved in maintaining basic cellular function. In contrast, COX2 is induced by physiological and pathophysiological stressors and plays important roles in the cellular response to stress (Herschman,1996 and Wadleigh *et al.*, 2000). Non steroidal anti-inflammatory drugs (NSAIDs) such as aspirin inhibit the COX enzymes, reducing inflammation. COX-1 is the constitutive isoform (Vane *et al.*, 1998), whereas COX-2 is the inducible isoform and considered to be a mediator of inflammatory disease (Venturini *et al.*, 1998). In addition to their role in inflammation, prostaglandins are important regulators of vascular tone, salt and water balance, and renin release, and nonselective NSAID exhibit adverse effects, including salt retention

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and reduced GFR, which may elevate BP or make pre-existing hypertension worse (Johnson, 1997).

Medullary prostaglandin E2 (PGE₂) plays an important role in regulating NaCl and water reabsorption in the medullary thick ascending limb and collecting duct (Stokes, 1981). Because COX-1 is abundantly and constitutively expressed in both cortical and medullary collecting duct, COX-1-derived prostanoids have been hypothesized to be involved in the natriuretic response, and in this regard, acutely increasing renal interstitial hydrostatic pressure by direct renal interstitial volume expansion will induce increased sodium excretion, which is blunted by infusion of nonselective NSAID but not COX-2 inhibitors (Gross, 1999).

Furthermore, in a rat model of cirrhosis and ascites, a COX-1 selective inhibitor but not a COX-2 selective inhibitor decreased sodium excretion and impaired the diuretic and natriuretic responses to furosemide (Lopez-Parra *et al.*, 2002). Recently, administration of selective COX-2 inhibitors or COX-2 gene knockout has been shown to accentuate the vasoconstrictor effects of angiotensin II in mice (Qi, 2002). Deterioration of renal function may also be observed in patients receiving non steroidal anti-inflammatory drugs (NSAIDs) or COX2 selective inhibitors (Whelton, 1999).

However, for the first time Folia (2005) observed that antihypertensive treatment which advantageously decreased the aggregability of erythrocytes was diminished by a high dose of aspirin (300 mg/day).

Results for celecoxib are somewhat different. One study found that cardiovascular side effects, such as myocardial infarction, stroke, or heart failure, were associated with prolonged treatment with celecoxib (Solomon *et al.*, 2005). In addition, Gupta *et al.* (2007) concluded that chronic administration of celecoxib may have a damaging effect on kidney, as evident through altered histopathology and renal functions. This damage may be mediated by oxidative stress. COX-2 is widely expressed throughout the kidney, and inhibition of this enzyme is contributory to reduced glomerular filtration, salt and water retention, and blood pressure elevation (Krum *et al.*, 2006). Thus, the aim of the current study was to investigate the hemodynamic and renal effects of COX -1 and COX-2 inhibitors in L-NAME induced hypertensive rats.

2. Materials and Methods

2.1. Animals and Housing

Twenty four adult female albino rats were used in this study. Female rats were used, so few studies are done on females because they have resistance in some models of hypertension rather than L-NAME-induced hypertension. All rats were weighing about (240 - 280 gm) and (7-9) weeks of age at the time when the experiment was started. Animals were housed in plastic cages bedded with wooden chips. They were housed under standard laboratory conditions, 12:12 light/dark photoperiod at 22 ± 2 °C. The animals were given standard rat pellets and tap water *ad libitum*.

2.2. Experimental Design

The present experiment was planned to study the possible hemodynamic, renal and liver effects of COX-1 and COX-2 inhibitors in L-NAME induced hypertensive rats. The experimental rats were divided into four groups, each with six animals and the treatments were continued for 2 weeks as follows:

Group 1: Control. The rats were given standard rat chow and tap water *ad libitum*.

Group 2: L-NAME. The rats were given standard rat chow and L-NAME at dose (20mg/kg body weight).

Group 3: COX-1 inhibitor (Aspirin). The rats were supplied with standard rat chow with aspirin (1000 mg /kg diet) and L-NAME at dose (20mg/kg body weight).

Group 4: COX-2 inhibitor (Celecoxib). The rats were supplied with standard rat chow with celecoxib (1000 mg /kg diet and L-NAME at dose (20mg/kg body weight).

2.3. Blood Pressure and Heart Rate Measurements

Measurements of SBP and heart rate were obtained at the end of the experiment by the tail-cuff method in all groups using power Lab (AD Instruments, power lab 2/25). During the week before treatment the rats were trained to become accustomed to the blood pressure measurements. Rats were placed in a restraining chamber and warmed to an ambient temperature of approximately 37°C, typically taking about 10-15 minute after that occluding cuffs and pneumatic pulse transducers were placed on the rats' tails. Six readings were taken for each rat, the highest, lowest and any associated with excess noise or animal movement were discarded. The average was taken of the remaining readings to generate a value for a given rat for that week.

2.4. Biochemical Determination Determination of Serum AST and ALT Activity

Serum AST and ALT activities were determined by colorimetric method kit ((Biolabo . SA, France)

2.5. Determination Of Serum Creatinine Level

Creatinine level was determined by colorimetric method kit (Biolabo . SA, France). Creatinine in alkaline picric acid solution, forms a color complex in which the absorbance was measured at 490 nm using spectrophotometer

2.6. Determination of Serum Uric Acid Level

Serum uric acid level was determined by uricase method, using colorimetric test kit (Biolab, France)

2.7. Determination of Serum Total Protein Level

Serum total protein level was determined by biuret method, using colorimetric test kit (Biolab, France)

2.8. Determination Of Serum Sodium And Potassium Ion Concentrations

Serum Na⁺ and K⁺ ion concentrations were determined by using flame photometer (Jenway Flame Analyzer, USA)

2.9. Serum Total Calcium Ion Determination

Ca²⁺-Kit enables colorimetric determination of total calcium (Chawla, 2003), without deproteinization. In serum, the calcium kit reacts with methylthymol blue

indicator (MTB) in an alkaline medium. The color intensity of the Ca^{2+} -MTB complex, measured at 612 nm, is proportional to the quantity of calcium present in the sample. The kit was obtained from (BIOLABO.SA, France).

2.10. Statistical Analysis

All data were expressed as means ± standard error (SE) and statistical analysis was carried out using available statistical soft ware (SPSS version 15). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons among groups were done using Duncan post hoc analysis. P values <0.05 were considered as significant.

3. Results

Figure 1 show the changes in SBP and H.R in L-NAME group rats. As expected, the SBP was elevated from 112.6 ± 2.752 mm Hg in control rats to 166.8 ± 2.561 mm Hg in L-NAME group . H.R was also raised from 353.6 ± 5.420 bpm to 397.6 ± 15.33 bpm .In the group of receiving COX-1 inhibitor, SBP significantly reduced to 144.8 ± 6.335 mm Hg ,while COX-2 inhibitor reduced it but not significantly. H.R was also changed after COX-2 inhibitor administration ,while COX-1 inhibitor did not change it significantly (Figure 2). Cox-1 administration increased serum Na^+ levels ,while serum K^+ levels was significantly increased in COX-2 group rats(6.829 ± 0.2892 mmole/L) compared with the L-NAME group (5.619 ± 0.2511 mmole/L).Neither COX-1 nor COX-2 administration caused change in serum Ca^{2+} shown in Table 1.

Supplementation with 30 mg/100ml drinking water of L-NAME for 15 days produced a significant increase in serum AST (86.64 ± 2.678 IU/L) compared with the control group(68.67 ± 4.3201 IU/L). AST (IU/L) did not change by COX-1 and COX-2 supplementation.(Table 2).Statistical analysis revealed that a significant reduction in ALT(IU/L) was observed by COX-2 administration (25.82 ± 3.392) compared with the L-NAME group (38.77 ± 1.3882). No significant change in serum ALT was obtained by in COX-1 group rats. The reduction of serum ALP (IU/L) that was detected by L-NAME administration was significantly returned to the control group by COX-2 inhibitor, while COX-1 inhibitor did not change it significantly.

As illustrated in figure 1,COX-2 inhibitor markedly elevated serum creatinine (0.942 ± 0.061 mg/dL) compared with the L-NAME group (0.679 ± 0.027 mg/dL).On the other hand, serum uric acid (mg/dL) was statistically increased in L-NAME group (1.9235 ± 0.362).There were no statistical difference in serum total protein among the studied groups.

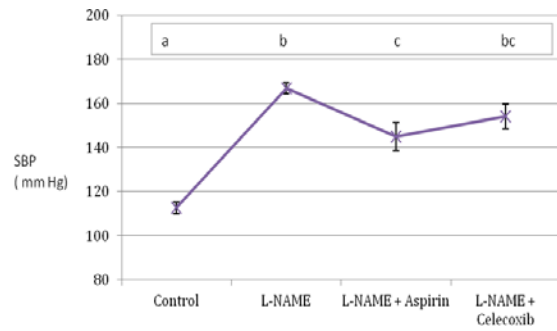


Figure 1. Effects of aspirin and celecoxib on SBP

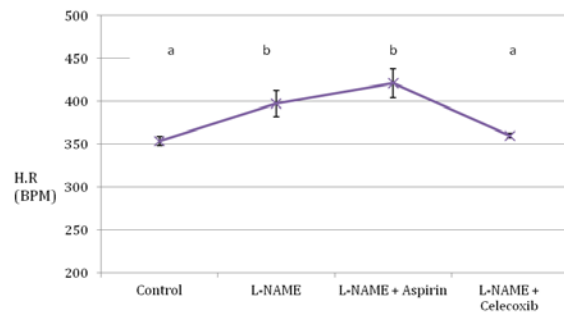


Figure 2. Effects of aspirin and celecoxib on serum H.R

Table 1. Effects of aspirin and celecoxib on serum Na^+ , K^+ and total Ca^{2+}

Parameters/Treatments	Control	L-NAME	L-NAME + Aspirin	L-NAME + Celecoxib
Serum Na^+ (mmol/L)	154.4 ± 2.673 ^b	146.9 ± 1.984 ^{ab}	157.2 ± 4.3054 ^b	137.3 ± 5.5614 ^a
Serum K^+ (mmol/L)	6.059 ± 0.3508 ^{ab}	5.619 ± 0.2511 ^a	6.299 ± 0.490 ^{ab}	6.829 ± 0.2892 ^b
Serum total Ca^{2+} (mg/dl)	6.2888 ± 0.378 ^a	6.6156 ± 0.442 ^a	5.6804 ± 0.161 ^a	6.7747 ± 0.339 ^a

The data presented as mean ± SE measured after 2 weeks of the treatments in all groups (Control , L-NAME(L-nitro-arginine methyl ester) , L-NAME + Aspirin (COX-1 inhibitor) , , L-NAME + Celecoxib (COX-2 inhibitor).

The same letters (a, a, a) mean non significant differences while the different letters (a, b, c) mean significant differences at $p < 0.05$

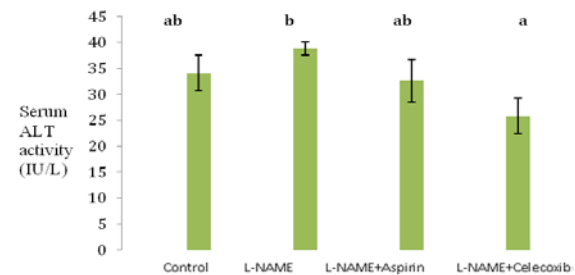


Figure 3. Effects of aspirin and celecoxib on serum ALT activity

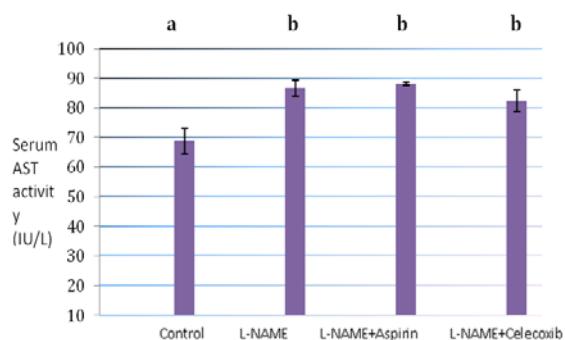


Figure 4. Effects of aspirin and celecoxib on serum AST activity

Table 2. Effects of aspirin and celecoxib on serum creatinine , uric acid and total protein levels

Parameters Treatments	Control	L-NAME	L-NAME + Aspirin	L-NAME + Celecoxib
Serum creatinine (mg/dL)	0.554±0.0342 ^a	0.679±0.027 ^{ab}	0.841±0.1374 ^{bc}	0.942 ± 0.061 ^c
Serum uric acid (mg/dL)	1.117±0.110 ^a	1.9235±0.362 ^b	1.1535±0.091 ^a	1.4517 ± 0.231 ^{ab}
Serum total protein (gm/dL)	5.741 ± 0.4486 ^a	6.388±0.314 ^{ab}	7.4917±0.625 ^b	6.1750 ± 0.325 ^{ab}

The data presented as mean ± SE measured after 2 weeks of the treatments in all groups (Control , L-NAME(L-nitro-arginine methyl ester) , L-NAME + Aspirin (COX-1 inhibitor) , L-NAME + Celecoxib (COX-2 inhibitor). The same letters (a , a , a) mean non significant differences while the different letters (a, b, c) mean significant differences at $p < 0.05$

4. Discussion

In the present study, results show that SBP was elevated by L-NAME for 2 weeks of administration at a dose of 30 mg/100ml in drinking water. As it was mentioned previously, L-NAME produce hypertension due to its elevation of ANGII (Navar *et al.*, 1996). L-NAME basically inhibit NOS synthase activity and reduce the level of NO (Oktem *et al.*, 2011). NO is a potent vasodilator (Vallance and Chan, 2000). Reduction of NO levels increases blood pressure due to overriding vasoconstrictors, or L-NAME increases epinephrine levels (Zanchi *et al.*, 1995), or due to an increase in oxidative stress (Saniz *et al.*, 2005). The precise mechanism by which oxidative stress increases blood pressure is not fully understood. One possible mechanism is that oxidative stress may increase some of the vasoconstrictors like endothelin-1 (ET-1), (Wedgwood and Black, 2005). ET-1 is a potent vasoconstrictor (Methew *et al.*, 1997), thereby causes hypertension. On the other hand other investigators resulted that NO deficiency causes accumulation of superoxide anion or hydrogen peroxide which may have prohypertensive actions (Shokoji *et al.*, 2003). ET-1 also increases H.R as we obtained from the current results shown in Table 1.

Statistical analysis revealed that COX-1 inhibitor caused a significant reduction in SBP, but it could not returned to the control group. However, COX-2 reduced SBP but the degree of reduction did not reach the significant values. The possible mechanism of COX-1

effects may be due to reducing some of the prohypertensive effects of prostaglandins especially prostaglandin F2 and Thromboxane A2 (Kim *et al.*, 2010). On the other hand, acetylsalicylic acid reduce vascular O_2^- production through lowering nicotinamide diphosphate hydrogen oxidase activity (Rong *et al.*, 2002). As previously mentioned, free radicals increases blood pressure (Shokoji *et al.*, 2003). Natriuretic effects of COX-1 inhibitor also may be different factors to reduce SBP. Furthermore, recently, Zhang *et al.* (2007) observed that aspirin reverses adrenocorticotrophic (ACTH) hormone. ACTH releases cortisol, and the water and salt retention increases consequently blood pressure will raises. However, COX-2 inhibitor reduced heart rate significantly, but such reduction did not affect systolic blood pressure and the effects of COX-2 on systolic blood pressure in the current results is consistent with Richter *et al.* (2004), in their experiment in renovascular hypertensive rats observed that blood pressure was not affected by COX-2 administration. In the recent study, Zaitone *et al.* (2011) observed that COX-2 did not affect blood pressure. However, the exact mechanism by which COX-1 and COX-2 affect hemodynamics remain to be explained.

As shown from the present results, serum Na^+ was slightly increased by COX-1. On the other hand, COX-2 markedly elevated serum K^+ level compared with the L-NAME group rats. The same results regarding COX-1 related with serum Na^+ regulation was obtained by Lopez-Parra *et al.* (2002), they showed that COX-1 inhibitor but not a COX-2 selective inhibitors decreased sodium excretion and impaired the diuretic and natriuretic response to furosemide. It is well known that reduction of Na^+ excretion will elevate its values in the serum. However, our result (Effects of COX-2 on serum Na^+ level) in this point is in contrast to Richter *et al.* (2004), they concluded that celecoxib treatment did not alter serum sodium in two kidney-one clip renovascular hypertensive rats.

The current results also revealed that COX-2 inhibitors significantly elevated serum K^+ level. One possible mechanism is that COX-2 inhibitors which may have a damaging effect on kidneys (Gupta *et al.*, 2007) may reduce K^+ secretion, as a result serum K^+ levels increases. According to the present results regarding effects of COX-1 and COX-2 inhibitors on serum Ca^{2+} level, both of these enzyme inhibitors may have not associate with parathyroid hormone, calcitonin and vitamin D metabolism, otherwise serum Ca^{2+} in contrast to the obtained results be reduced or increased compared with control group.

The enzymatic activity of AST and ALT were studied to estimate liver and heart malfunctions. As shown in figure (4), serum AST activity was significantly increased after L-NAME administration. Previous researchers observed that serum AST activity increased after myocardial infarction and hepatic parenchymal injuries (Burtis and Ashwood, 1994). Serum ALT activity was also elevated in group of L-NAME. Sainz *et al.* (2005) showed that hypertension produced by NOS inhibitor is significantly related with oxidative stress. Free radicals

included cell damage may be determined by MDA concentration (Oktem *et al.*, 2011). Deniz *et al.* (2006) observed that NOS inhibitor induced hypertension increases malonaldehyde concentration. Other possible causes of AST and ALT activities elevation by L-NAME may be related with an increase of some vasoconstrictors such as ANG II and ET-1, which may they enhance free radical formation (Schnackenberg, *et al.*, 2000; Touyz, 2003).

Neither COX-1 nor COX-2 inhibitors ameliorate serum AST activity, whereas serum ALT activity slightly reduced by COX-1 and significantly ($P < 0.05$) reduced by COX-2 inhibitor. Renna *et al.* (2009) concluded that chronic aspirin treatment normalized relative heart weight and vascular remodeling. Furthermore, Washino *et al.* (2010) according to their results suggested that celecoxib effectively ameliorates the necrotic action and the oxidative stress induced by tetrachloroacetic acid CCL4. Ozturk *et al.* (2006) also confirmed the beneficial effect of celecoxib in hepatic ischemias reperfusion injury and they suggested that celecoxib may protect the liver. On the other hand, Malek and Saleh (2009) resulted that celecoxib produce significant reduction in serum ALT activity, MDA and alpha tumor necrotic factor.

Results of the present study shows that COX-2 inhibitor markedly increased serum creatinine compared with the L-NAME group. Gupta *et al.* (2007) obtained the same result showing chronic administration of celecoxib alter renal functions. In another study, patient receiving COX-2 selective inhibitors, deterioration of renal function may be observed (Whelton, 1999). However, this elevation of creatinine level by COX-2 in our study is in contrast to Richter *et al.* (2004), they resulted that celecoxib did not alter GFR and serum creatinine. The present results also showed that L-NAME administration significantly raised serum uric acid. Reduction of NO levels elevate Ang II and ET-1 which represent key mediators for kidney damage (Amann *et al.*, 2001; Dhaun *et al.*, 2006), consequently serum uric acid may from the blood (Kumar *et al.*, 2000). In conclusions, the results suggested that aspirin rather than celecoxib reduces SBP and in contrast to aspirin, celecoxib alter kidney functions through elevation of serum creatinine level, but it may attenuate liver functions through reduction of elevated serum ALT activity by L-NAME administration.

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