An Interaction Study between Acetylsalicylic Acid (Aspirin) and Captopril at Different Doses in Rats

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Abstract

The present studies were used forty nine rats to determine chronic toxicity effect which were divided into 4 groups, the control group (7 rats) received distilled water, the other three treatment groups each group (14) rats were divided into two subgroups T1&T2 according to the following oral daily dosing regiment (aspirin subgroup 2 and 4 mg/kg , captopril, 6 and 12 mg/kg and aspirin + captopril (2 + 6 mg/kg and (4+12 mg/kg) respectively.

The following biochemical and hematological results were recorded during the experiment:
Prothrombine Time : Captopril, aspirin and captopril + aspirin treated rats showed significant increase ($P < 0.05$) in serum PT levels in both T1&T2 subgroups at the period of 1.5 & 3 months treatment positively proportional with the dose and treatment period.
Clotting Time: No significant changes were noticed in clotting time of aspirin groups and captopril treated rat groups, while the combined treated subgroups T1&T2 showed a significant increase after 3 month.
Blood Urea Nitrogen :Captopril, aspirin and captopril + aspirin treated rats showed significant increase ($P < 0.05$) in serum BUN levels in both T1&T2 subgroups at the period of 1.5 & 3 months treatment positively proportional with the dose and treatment period.
Total Body weight : Significant increase in body weight were observed in captopril, aspirin and captopril + aspirin treated rats in both T1&T2 subgroups through out the experimental periods proportional to the do……..se and treatment periods.

الخلاصة

بمختلف الجرع في الجرذان استخدمت تسعة وأربعون جرذا فقد قسّمتْ إلى 4 مجاميع ،أعطيت مجموعة السيطرةُ(7 جرذ) ماءاً مُقَطَّراً أما المجموعاتَ المعالجة الثلاثة مجموعات كل منهما 14 جرذ قسّمتْ إلى مجموعتين فرعيتين T1 و T2 كل منهما7 جرذ جرعت فمويا يوميا ولمدة 3 أشهر أسبرين 2و4 ملغم / كيلوغرام وكابتوبريل 6و12 ملغم /كيلوغرام وأسبرين+ كابتوبريل (2+6 ملغم / كيلوغرام)،(4+12 ملغم /كيلوغرام) على التوالي.
سُجّلتْ أثناء التجربةِ النتائج الكيماحيوية والفسلجية التالية:
وقت البروثرومبين (PT) : كانتَ زيادة كبيرة معنوية $P<0.05$ في جرذان المعالجة بالكابتوبريل والأسبرين ومزيجهما الأسبرين+ الكبتوبريل في كلا المجموعتين T_1و T_2 في وقت البروثرومبين خلال مدةِ 1.5 و3 شهور من العلاج مع الجرعة ومدةِ المعالجة.
وقت التخثر الدم (CT): لم يحدث أي تغيير معنوي $P<0.05$ في وقتِ التَخَثُر بعد المعاملة المزمنة بالكابتوبريل والأسبرين لمجموعتيT1 و T2 بينما ظهرت زيادة معنوية في مجاميع مزيجهما بعد ثلاثة شهور من العلاج
نتروجين يوريا الدم (BUN):وجدتَ زيادة معنوية $P<0.05$ في تركيز مستوى نتروجين يوريالدم (BUN) جرذان المعالجة بالكابتوبريل والأسبرين ومزيجهما الأسبرين+ الكبتوبريل في مستوى مصل BUN كلا مجموعتي T_1و T_2 في مدة فترةِ 1.5 و3 شهور من العلاج مع الجرعة ومدةِ العلاجَ في كل مجاميع التجربة.
وزن الجسم (B.W.) : سجلت زيادة معنوية في وزنِ الجسمَ للجرذان المعالجة بالكابتوبريل والأسبرين ومزيجهما الأسبرين+ الكبتوبريل في كلا مجموعتي T_1و T_2 خلال مدة 1.5 و3 شهور من العلاج بصورة إيجابية مع الجرعة ومدةِ العلاج.

Introduction

Acetylsalicylic acid (aspirin) in the 1980s, its ability to inhibit platelet
aggregation was realized and it became an important antithrombotic agent[1] .
In the late 1960s to the early 1970s stated they believed an ACE inhibitor would be herapeutically useful in the treatment of so-called "essential" hypertension[2].

The mechanism for an acetylsalicylic acid - angiotensin-converting enzyme inhibitor interaction is believed to be pharmacologic rather than pharmaco-kinetic. Dosages of acetylsalicylic acid of 100 mg/day or less seem to interact little with angiotensin converting enzyme inhibitors, whereas higher dosages may carry a higher risk, and some patients, for whatever reason, may be more susceptible to the interaction[3]. Acetylsalicylic acid (160 to 325 mg) should be given as soon as possible to all patients with acute myocardic infarction [4].

Aims

Monitoring of the effects of acetylsalicylic (aspirin) acid and angiotensin-converting enzyme inhibitor (captopril) administered on body weight changes, hematological parameters in different orally dosed rat groups and effects on Prothrombin, Clotting times and blood urea nitrogen.

Materials and Methods

Forty nine adult male rats were divided into four groups; first group (7 rats) was act as control receive distilled water orally , the second group(14 rats) received captopril daily and divided equally into two subgroups T1 and T2 dosed daily orally with a maximum therapeutic dose 6 mg/kg and 12 mg /kg respectively for 3 months.

The third group (14 rats) received aspirin daily and divided equally into two subgroups T1 and T2 .T1 were given orally 2 mg /kg while T2 were administrated orally 4 mg /kg .The fourth group (14 rats) received captopril and acetylsalicylic acid daily and divided into two subgroups T1 and T2 .T1(7 rats) were given orally (6 + 2) mg / kg while T2 (7 rats) were given orally (12 + 4) mg /kg, captopril and acetylsalicylic acid respectively once time a day for 3 months. (Figure1)

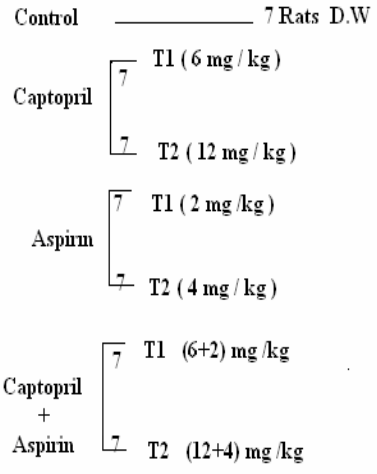

Figure 1. Groups and Subgroup Treatment For Acute And Chronic Toxicity Studies

Blood Sampling

Each animal was anaesthetized by chloroform then 4 ml of blood was withdrawn directly from the heart at different intervals along the experiment using sterile disposable syringe needle (Gage 23), Then 1.8 ml of blood WAS added to a tube containing 0.2 ml of sodium citrate for prothrombin time test .The remaineder of the blood was left for half an hour to stand to allow for clot formation and then centrifuged at 4000 rpm for 15 minute, The serum obtained was used immediately for the hematological and biochemical tests and according to experimental periods baseline, 1.5 months and 3 months.

Hematological test:

Prothrombin Time:

A blood volume of 1.8 ml was withdrawn from the heart and added to a tube containing 0.2 ml sodium citrate and mixed well and centrifuged. for 10 minutes, Then it was incubated for 15 minutes at 37C°, from which 0.1 ml serum to tube contain 0.1 ml kit thromboplastin kit in a water bath at 37C°observed each second till white small balls appears. By counting the time of their appearance, prothrombin time was measured per second, according to Coles [5].

3.5.1.2.Clotting Time:

Non heprinized capillary tube (Micro-Haematocrit Tubes) was used for blood drawn from the tail. Timing begins when blood is first collected and ends when a fibrin strand appears. A piece of the tube was cut each minute, By counting the number of pieces, clotting time were measured per minute [5].

Blood urea nitrogen:

According to the Berthelot reaction, the enzyme urase converts urea by hydrolysis to carbonic acid and NH3. The ammonia formed reacts with phenol in the presence of hypochlorite to form indophenols which in alkaline medium gives a blue colour compound. Nitroprusside acts as a catalyst, increasing the rate of reaction, the intensity of the colour obtained and its reproducibility was measured spectro-photometrically [5].

The reactions are:

$$\text{Urea} + H_2O \xrightarrow{\text{Urase}} Co_2 + 2NH_3$$

$$NH3 + HOC1 \longrightarrow NH2\ C1 + H2O$$

$$NH2\ C1 + C6\ H5\ OH \longrightarrow O{=}\ C6\ H4 = NC1$$

$$O{=}C6H4{=}NC1 + C6H5OH \longrightarrow C{=}C6\ H4 = N\text{-}C6\ H4\ C^{-}$$

Calculation blood urea was dose according to the following equation

$$\text{Serum blood urea nitrogin (mmol/L)} = \frac{\text{Test}}{\text{Standard}} \times 20$$

Animal weight

Animal weight were measured and monitored weekly through all the experimental period for all groups and subgroups.

Results

Prothrombine Time : Captopril, aspirin and captopril + aspirin treated rats showed significant increase ($P < 0.05$) in serum PT levels in both T1&T2 subgroups at the period of 1.5 & 3 months treatment positively proportional with the dose and treatment period.

Table1. Effect of different doses of captopril on plasma prothrombin time (second) in different experimental groups & treatment periods

Group / period	Pretreatment Mean ± SE	1.5 months Mean ± SE	3 months Mean ± SE
Control n=7	Aa 13.0 ± 0. 6	Aa 14. 0 ± 0. 6	Aa 13.3 ±1. 7
T1 n=7	Aa 13.0 ± 0. 6	Bb 18.3 ± 0. 9	Bb 21.3 ±1.9
T2 n=7	Aa 12.90 ±1. 2	Bb 19.7 ± 0.8	Bb 23.0 ± 0.6

T1 = 6 mg/kg B.W.
T2 = 12mg/kg B.W.
Different capital letters means, there are significant differences within groups at P<0.05.
Different small letters means, there are significant differences between groups at P <0.05.
n= number of animal

Table 2. Effect of different doses of aspirin on the plasma prothrombin time (second) in different experimental groups & treatment periods

Group / period	Pretreatment Mean ± SE	1.5 months Mean ± SE	3 months Mean ± SE
Control n=7	Aa 13.0 ±0. 6	Aa 14. 0 ±0. 6	Aa 13.3 ±1. 7
T1 n=7	Aa 12. 7±1.01	Bb 20.0 ±0.00	Bb 21.0 ±0.6
T2 n=7	Aa 12. 7 ±1.20	Bb 23.3 ± 0.9	Bb 25.0 ±1.2

T1 = 2 mg/kg B.W.
T2 = 4 mg/kg B.W.
Different capital letters means, there are significant differences within groups at P < 0.05.
Different small letters means, there are significant differences between groups at P <0.05.
n= number of animal

Table 3. Effect of different doses of aspirin and captopril on the plasma prothrombin time (second) in different experimental groups & treatment periods

Group/ period	Pretreatment Mean±SE	1. 5 months Mean±SE	3 months Mean±SE
Control n=7	Aa 13.0 ± 0. 6	Aa 14. 0 ± 0. 6	Aa 13.3 ± 1. 7
T1 n=7	Aa 13.3 ± 0.4	Bb 19.0 ± 2.3	Bb 22.0 ± 1.20
T2 n=7	Aa 13.0 ± 1. 2	Bb 22.0 ± 0.3	Bb 24.0 ± 0. 6

T1 = (captopril 6 + aspirin 2) mg/kg B.W.
T2 = (captopril 12 + aspirin 4) mg/kg B.W.
Different capital letters means, there are significant differences within groups at P<0.05.
Different small letters means, there are significant differences between groups at P <0.05.
n= number of animal

Clotting Time:

No significant changes were noticed in clotting time of aspirin groups and captopril treated rat groups, while the combined treated subgroups T1&T2 showed a significant increase after 3 month. (Table 4,5,6).

Table 4. Effect of different doses of captopril on the blood clotting time of different experimental groups & treatment periods

Group / period	Pretreatment Mean ± SE	1.5 months Mean ± SE	3 months Mean ± SE
Control n=7	3. 2 ± 0.4	3. 2 ± 0.4	3.0 ± 0. 6
T1 n=7	3.0 ± 0. 3	3. 7 ± 0.3	3.0 ± 0. 6
T2 n=7	3. 2 ± 0.60	3.50 ±0. 3	3.0 ± 0. 6

T1 = 6 mg/kg B.W.
T2 = 12 mg/kg B.W.
n= number of animals

Table 5. Effect of different doses of aspirin on the blood clotting time of different experimental groups & treatment periods

Group/period	Pretreatment Mean ± SE	1.5 months Mean ± SE	3 months Mean ± SE
Control n=7	Aa 3. 2 ±0.4	Aa 3. 2 ±0.4	Aa 3.0 ±0. 6
T1 n=7	Aa 3.3 ±0. 5	Aa 4.0 ±0. 3	Aa 4.2 ± 0.4
T2 n=7	Aa 3.3 ±0.4	Aa 4.0 ±0. 6	Aa 3.5 ± 0.8

T1 = 2 mg/kg B.W.
T2 = 4 mg/kg B.W.
n= number of anima

Table 6. Effect of different doses of aspirin and captopril on the clotting time of different experimental groups & treatment periods

Group/ period	Pretreatment Mean±SE	1. 5 months Mean±SE	3 months Mean±SE
Control n=7	Aa 3. 2 ±0.4	Aa 3. 2 ±0.4	Aa 3.0 ±0. 6
T1 n=7	Aa 3.3±0.7	Aa 2.50 ±0. 3	Bb 5. 7 ±0.3
T2 n=7	Aa 3.3 ±0. 9	Aa 2. 2 ±0. 2	Bb 4. 8 ±0.7

T1 = (captopril 6 + aspirin 2) mg/kg B.W.
T2 = (captopril 12 + aspirin 4) mg/kg B.W.
Different capital letters means, there are significant differences within groups at $P<0.05$.
Different small letters means, there are significant differences between groups at $P<0.05$
n= number of animals

Blood Urea Nitrogen:

Captopril, aspirin and captopril + aspirin treated rats showed significant increase ($P< 0.05$) in serum BUN levels in both T1&T2

Table 7. Effect of different doses of captopril on the serum blood urea nitrogen level (mmol/l) of different experimental groups & treatment periods

Group / period	Pretreatment Mean ± SE	1.5 months Mean ± SE	3 months Mean ± SE
Control n=7	Aa 9. 2 ± 1. 6	Aa 10.5 ± 1.05	Aa 8.50 ± 1.05
T1 n=7	Aa 7.4 ± 0. 3	Bb 21.7 0.9	Bb 19.8 ± 0.6
T2 n=7	Aa 10.2 ± 1.60	Bb 23.0 0.6	Bb 20.8 ±0.8

T1 = 6 mg/kg B.W.
T12 = 12 mg/kg B.W.
Different capital letters mean there are significant differences within groups at P<0.05.
Different small letters mean there are significant differences between groups at P <0.05.
n =number of animals

Table 8. Effect of different doses of aspirin on the serum blood urea nitrogen level (mmol/l) of different experimental groups & treatment periods

Group / period	Pretreatment Mean ± SE	1.5 months Mean ± SE	3 months Mean ± SE
Control n=7	Aa 9. 2 ±1. 6	Aa 10.5 ±1.05	Aa 8.50±1.05
T1 n=7	Aa 8.5 ±0.30	Bb 19.6± 0.5	Bb 23.5±0.5
T2 n=7	Aa 9.6 ±0.5	Bb 20.1 ± 0.5	Bb 25.06 ±1.0

T1 = 2mg/kg B.W.
T2 = 4 mg/kg B.W.
Different capital letters mean, there are significant differences within groups at P<0.05.
Different small letters mean, there are significant differences between groups at P <0.05.

Table 9. Effect of different doses of aspirin and captopril on the serum blood urea nitrogen level (mmol/l) of different experimental groups & treatment periods

Group/ period	Pretreatment Mean ± SE	1. 5 months Mean ± SE	3 months Mean ± SE
Control n=7	Aa 9. 2 ±1. 6	Aa 10.5 ±1.05	Aa 8.50±1.05
T1 n=7	Aa 8.1 ±0.4	Bb 17.7 ± 1.0	Bb 16.9 ± 0.8
T2 n=7	Aa 9.3 ±0.7	Bb 21.3 ±1.6	Bb 19.2 ± 1.4

T1 = (captopril 6 + aspirin 2) mg/kg B.W.
T2 = (captopril 12 + aspirin 4) mg/kg B.W.
Different capital letters mean, there are significant differences within groups at P<0.05.
Different small letters mean, there are significant differences between groups at P <0.05.

Total Body weight:

Significant increase in body weight were observed in captopril, aspirin and captopril + aspirin treated rats in both T1&T2 subgroups through out the experimental periods proportional to the dose and treatment periods (Table – 10,11,12).

Table 10. Different monthly animal total body weight (grams) for captopril dosed groups at different experimental periods

Group/period	Pretreatment Mean ± SE	1 month Mean ± SE	2 months Mean ± SE	3months Mean ± SE
Control n=7	Aa 200.0 ± 5.77	Aa 216.0 ±5.0	Aa 220.7± 6.0	Aa 225.8±6.1
T1 n=7	Aa 195.0 ± 5.5	Aa 210.0 ± 6.5	Ba 235.0±7.3	Ba 254.1±8.6
T2 n=7	Aa 196.7 ± 16.2	Aa 214.2 ± 7.2	Ba 246.8 ± 3.8	Ba 263.3±9.8

T1 = 6 mg/kg B.W.
T12 = 12 mg/kg B.W.
Different capital letters mean, there are significant differences within groups at P<0.05.
Different small letters mean, there are significant differences between groups at P <0.05
n= number of animal

Table 11. Different monthly animal total body weight (grams)for aspirin dosed groups at different experimental periods

Group/period	Pretreatment Mean ± SE	1 month Mean ± SE	2 months Mean ± SE	3months Mean ± SE
Control n=7	Aa 200.0 ± 5.77	Aa 216.0 ±5.0	Aa 220.7± 6.0	Aa 225.8 ± 6.1
T1 n=7	Aa 214.2 ± 16.0	Aa 230.0 ±9.8	Bb 257.5 ±10.9	Bb 277.8 ±5.8
T2 n=7	Aa 192.5± 10.5	Aa 211.7 ±10.9	Bb 248.3 ±10.3	Bb 270.0 ±12.5

T1 = 2mg/kg B.W.
T2 = 4 mg/kg B.W.
Different capital letters mean, there are significant differences within groups at P<0.05.
Different small letters mean, there are significant differences between groups at P <0.05
n= number of animal

Table 12. Different monthly animal total body weight (grams)for experimental groups of interaction (captopril and aspirin) at different treatment periods

Group/ period	Pretreatment Mean ± SE	1 month Mean ± SE	2 months Mean ± SE	3months Mean ± SE
Control n=7	Aa 200.0 ± 5.77	Aa 216.0 ±5.0	Aa 220.7 ±6.0	Aa 225.8 ± 6.1
T1 n=7	Aa 190.8 ± 5.0	Aa 219.2 ±2.5	Bb 243.3 ±5.8	Bb 265.0 ±13.7
T2 n=7	Aa 190.3 ± 10.9	Aa 227.5 ±4.2	Bb 259.2 ±10.6	Bb 266.7 ±9.1

T1 = (captopril 6 + aspirin 2) mg/kg B.W.
T2 = (captopril 12 + aspirin 4) mg/kg B.W.
Different capital letters mean, there are significant differences within groups at P<0.05.
Different small letters mean, there are significant differences between groups at P <0.05
n= number of animal

Discussion

Prothrombin time:

In the present study, a significant increase in prothrombin time was found in captopril groups (T1&T2) at 1.5& 3 months period in comparison with the control group and pretreatment period. This finding is similar to that repotted by Zelezniakowicz, *et al.*,(2006)[6] that ACEIs caused inhibition in platelet adhesion, aggregation and prolongation of prothrombin time in rats. By contrast, Buczko, *et al.,* (2004)[7]. found that none of these drugs changed prothrombin time.

A significant increase with a little higher prothrombin time level reported in aspirin treated group animals for the same period. This finding is similar to that recorded by Szczeklik, *et al.,* (1996) [8] who indicate that the effects of aspirin on thrombin generation occur with low-dose aspirin treatment.

Aspirin markedly prolonged the time at which thrombin reached its peak clotting activity positively proportional with the dose and treatment period. Since the precise mechanism of the inhibitory action of aspirin on thrombin generation is not known, to think that suppression of thrombin generation by aspirin results rather from acetylation of macromolecules of platelet membranes and/or of prothrombin than from the inhibition of platelet cyclooxygenase (Szczeklik, *et al.,* 1996)[8].

A single dose of aspirin depresses the rate of thrombin generation, while chronic treatment reduces the total amount of thrombin generated. High doses of aspirin are also given immediately after an acute heart attack. These doses may also inhibit the synthesis of prothrombin and may therefore produce a second and different anticoagulant effect (Tohgi, *et al.,* 1992)[9].

The same significant increase was found after aspirin and captopril combination treatment. This finding is similar to Patrick and Kamath, (1996)[10].

Clotting Time:

In the present study, it was noticed that there were no significant changes in clotting time for all experimental groups and periods after captopril treatment. This finding is similar to that of Ewa Chabielska1, *et al.,* (2005) and Buczko, *et al.,* (2004) [7,11]. Also this study showed a little increase but not significant in clotting time of aspirin T1 and T2 groups. This finding is similar to the finding of Fabio, (2004)[12]. That aspirin's anti-platelet action declines with prolonged treatment. Despite aspirin's demonstrated effectiveness in treating and preventing atherosclerotic disease, it produces only partial inhibition of platelet aggregation, and therefore it is a relatively weak antiplatelet agent. while their interaction caused significant increase in T1 & T2 groups after 3 months treatment in comparison with that of control group and experimental periods may be attributed to aspirin inhibition of298 platelet aggregation. The mechanism of antithrombotic action of this drug seems to be dependent on the suppression of coagulation cascade and the enhancement of the fibrinolytic processes**.**

Blood Urea Nitrogen:

In our study we found that captopril causes a significant increase in serum BUN levels in both T1 & T2 groups in the 1.5& 3 months period that may be attributed to the adverse effect of captopril on the kidney which may causes renal failure in large dose therapy for long period Also significant increases in BUN were noticed for the effect of different doses of aspirin. This finding is similar to that of Segal, *et al.,* (2006) [13]. who noticed such increases in patients taking aspirin therapy for long period. These result may be either due to true decrease in BUN clearance or alternatively a change in its tubular

handling. Another hypothetical mechanism may be attributed to aspirin-induced hypovolemia and extracellular volume changes reflected by the significant BUN changes in patient, Therefore it is recommended to avoid the use of aspirin in severe renal failure patient. Chronic dosing with both drugs caused significant increase in the serum BUN levels due to their additive effects (Segal, *et al.,* 2006)[13].

Body weight:

In the present study a significant increase in the body weight of animal experimental groups were noticed through out the experimental period after different doses of captopril. This finding is similar to that of King, *et al.,* (2006)[14] who found that Benazepril increases food intake and water body weight in healthy growing cats. This increase may be attributed also to the large captopril dose and long treatment period due to the effect of prostaglandin leading to increased fat tissue (Fetliawi, 2005)[15].

Significant increase in the body weight was seen with different doses of aspirin which could be attributed to the increased food and water intake. This may be due to the irritant effect of acidic aspirin on the stomach mucosa.

Aspirin and NSAIDs sometimes affect the normal function of the kidneys and aspirin-like drugs promote the retention of salt and water by reducing the prostaglandin-induced inhibition of both the reabsorption of chloride and the action of antidiuretic hormone. This may cause edema in some patients with arthritis who are treated with an aspirin-like drug. (Murray and Brater,1993)[16].A significant increase in the body weight according to the dose and treatment period was found after both aspirin and captopril treatment, This may be due to their additive effect, through Opposing the production of angiotensin II by ACE inhibition which may result in vasodilation, in part by the attenuation of thromboxane-A_2 production. Since aspirin inhibits COX and thereby thromboxane A_2 production, the two agents may act synergistically (Schwartz, *et al.,* 1992)[17]. Thus, the inhibition of ACE increases bradykinin levels with the consequent enhanced synthesis of vasodilatory prostaglandins, appear to mediate a significant benefit of ACE inhibitor therapy in these patients (Gainer, *et al,* 1998)[18].

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