Acute and Chronic Interaction Study BetweenAcetylsalicylic Acid (Aspirin) and Captopril atDifferent Doses

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دراسة التفاعل الحاد والمزمن بين الأسبرين والكابتوبرل بمختلف الجرع في الجرذان

الخلاصة:

أجريت هذه التجربة بهدف تحديد السمية الحادّةِ LD50 والمُزمنةِ للأسبرين والكابتوبرل ومزجهما بعد التجرع لمجاميع جرذانٍ معاملة.

أستخدمت سبعون جرذا منها ٢١ لأحداث التسمم الحادِ لكل من الأسبرين والكابتوبرل ومزجهما معا بعدِ أعطائهم جرعةِ واحدة، أما الجرذان ٤٩ الباقية فقد قسّمتْ إلى ٤ مجاميع ،أعطيت مجموعة السيطرةُ(٧ جرذ) ماءاً مُقَطراً أما المجموعاتَ المعالجة الثلاثة مجموعات كل منها ١٤ جرذ قسّمتْ إلى مجموعتين فرعيتين T1 و T2 كل منها٧ جرذ جرعت فموياً ولمدة ٣ أشهر أسبرين ٢و٤ ملغم / كلوغرام وكابتوبرل ٦و١٢ ملغم /كلوغرام وأسبرين+ كابتوبرل (٢+٦ ملغم / كلوغرام)،(٤+١٢ ملغم /كلوغرام) على التوالي.

حددت الجرعة الوسطى الممتة في دراسة الحادة واصبحت للكابتوبرل والأسبرين° ٤.٣٧٠٥غم/ كلوغرام، ٥.٣٧٠٥غم/ كلوغرام على التوالي ، أما مزجهما فقد وسجلا النتائج التالية كابتوبرل ٢.١٢٩٥غم/ كلوغرام + أسبرين٢.٦٢٩٥ غم/ كلوغرام على التوالي أما نتائج التجربة المزمنة للأدوية أظهرت انخفضا في قيم بعد العلاج المُزمنِ بكل المجاميع وحسب المعاملة وكانت٣.١٣١٥ غم / كلوغرام ، للكابتوبرل ، ٣.١٢٩٥غم / كلوغرام للاسبرين ومزج كابتوبرل+ الأسبرين افقد سجل١.٦٣١٥ غم / كلوغرام + ٢.١٣١٥غم / كلوغرام على التوالي.

سجلت النتائج التالية للتداخل بين الدوائين بعد حساب قيم LD50 بطريقة Isobolographic كان تأثير أضافة دوائين بعد التعرض الحاد وتضاد التعرض المُزمنِ. ووجد أن هذين الدوائين لم يظهرا تأثير تراكمياً بعد ٣ أشهرٍ من المعالجِ اليومي وذلك حسب نتائج معامل التراكم لمجاميع المعاملة.

ستنتج في هذه الدراسة ان الاسبرين والكابتوبرل لهما علاقة أضافة Additive effect عند التعرض للتسمم الحاد وعلاقة التضاد Antagonism effect بعد التعرض للتسمم المزمن وليست تأثير تراكمي لكلا الدوائين في الجسم

Abstract

The present studies were conducted to determine acute and chronic toxicity of aspirin and captopril given alone or in combination in seventy rats were used, 21 rats for acute toxicity (LD50) of aspirin, captopril and their combination in single dose, while the other forty nine rats were used 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.

LD50 was measured for all groups after their chronic treatment to determine chronic index.

The results of acute toxicity (LD50) were, for captopril 4.3705 g/kg, aspirin 5.3705 g/kg and their combination (captopril 2.1295 g/kg + 2.6295 g/kg aspirin), respectively that reduced after chronic drug exposure to 3.1315 g/kg captopril, 3.1295g/kg aspirin and captopril 1.6315 g/kg + 2.1315 g/kg aspirin.

Isobolographic analysis of two drug LD50 showed that there were an additive effect after acute toxic exposure and antagonism after chronic one

No accumulation was reported between the two drugs and their combination after 3 month daily treatment according to the results of chronic index of all treated groups.

This study indicate that LD50 isobolographic analysis for captopril and aspirin showed an additive effect after acute exposure and antagonism after chronic administration and no accumulation effect for both drugs

Introduction

Aspirin (Acetylsalicylic acid (ASA)) remains one of the world's NSAID,s that most extensively used drug. Captopril is one of the first class of angiotensin converting enzyme inhibitors (ACEIs) antihypertensive agents, is a specific competitive inhibitor of angiotensin -converting enzyme (ACE) that primarily inhibit the rennin-angiotensin-aldosterone system [(1).]

Some evidence supports a clinically significant aspirin-ACE inhibitor interaction in patients with essential hypertension. Whereas potentially detrimental aspirin-ACE inhibitor interaction in patients with heart failure dates back to several years age. Among coronary artery disease patients with and without heart failure who are treated with ACE inhibitors, the use of aspirin in combination with captopril was associated with lower mortality than treatment without aspirin [(2)].

Both aspirin (acetylsalicylic acid) and captopril (ACE inhibitors) are often used concomitantly, especially in patients with both heart failure and ischaemic heart disease[(3)]. Several studies have suggested that aspirin may attenuate the beneficial effects of ACE inhibitors when given concomitantly to patients with one or more cardiovascular diseases [(2)]. During the last decade, several studies have suggested that acetylsalicylic acid could have a possible negative therapeutic interaction with angiotensin-converting enzyme inhibitors in patients with congestive heart failure [(4; 5)].

Aims

Acute & chronic studies in rat groups administered with large doses of captopril and with a therapeutic doses of aspirin alone and together.

Study of the possible mechanism of these acute & chronic interaction studies.

Materials and Methods

Acute toxicity (single lethal dose)

Twenty one males rats were divided into three group : first group (7 rats) were administered a different oral lethal doses of acetylsalicylic acid. the second group (7 rats) were administered a different oral lethal doses of captopril while the third group (7 rats) were administered a different oral lethal doses of acetylsalicylic acid and captopril and their LD50 were calculated according to up & down (Dixon method)[(6).]

Dixon method

This test calls for dosing individual animals in sequence singly at 24-hour intervals, with the initial dose set at "the toxicologist's best estimate of the LD50." Following each death (or moribund state) the dose is lowered; following each survival, it is increased, according to a prespecified dose progression factor. If a death follows an initial direction of increasing doses, of 10-20 % or a survival follows an initial direction of decreasing dose with the same ratio, three additional animals are tested following the same dose adjustment pattern and then testing is ended. described

in isobolograph is used. The LD50 is calculated using the following equation (Appendex-1)[(6)].

LD50 = Xf + K d
Xf = last dose administered
K = value from Table
D = difference between dose levels

Chronic toxicity

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. (Figure -1)

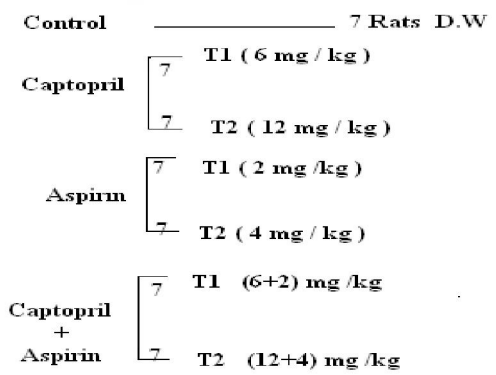

Figure- 1- Groups And Subgroup Treatment For Acute And Chronic Toxicity Studies

After treatment with oral doses for 3 months chronic toxicity symptom, possibility of drug accumulation and drug interaction were studied by using Isobolographic analysis. LD50 were conducted for all treatment groups at the end of experiment to determine their chronic index in comparison with their acute toxicity LD50.

Isobolographic analysis

This analysis usually used to determine the sort of interaction of two drugs, in which a line draw to join the LD50 of each of the two drugs studied in isobolograph and an intersection point determined by lines drawed vertically from the LD50 values of their interaction.

The conclusion of drugs interaction were determined accordingly to the position of this intersection point to the two drugs joined LD50 line. If the point is to the right, it mean there is an antagonistic effect, to the left, mean potentiation or synergistic effect, while on the line, it mean there is an additive effect between the two drugs.[7]

Chronic index

It is a measure of the possibility of a drug accumulation after its chronic administration based on comparison the ratio of LD50 values after acute and chronic exposure to the drugs according to this equation:

$$\text{Chronic index} = \frac{LD50 \text{ of single dose(acute)}}{}$$

LD50 after 3 months dose with sublethal dose

If the result exceed 2, there is possibility of accumulation .[8]

Results

Acute Toxicity Acute toxicity studies showed that LD50 for captopril and aspirin according to Dixon method were 4370.5 & 5370.5 mg/ kg that reduced by 50 % after their interaction to 2129.5 + 2629.5 mg/kg (Table -1-).

Isobolographic analysis of two drug LD50 showed that there were an additive effect after acute toxic exposure. (Figure -2-).

Table - 1- Estimate Of LD50 Dose For Captopril, Aspirin And Their Interaction In Acute Toxicity Study

Treatment / Groups	Initial dose mg/kg body weight	Final dose mg/kg body weight	Number of animal	Result after 24 hours	LD50 Mg/kg
Captopril	3000	4000	7	OOOXOXO	4370.5
Aspirin	4000	5000	7	OOOXOXO	5370.5
Captopril + Aspirin	3500 + 4000	2500 + 3000	7 + 7	XXXOXOX	2129.5 + 2629.5

O = survival Animal **X= dead animal**

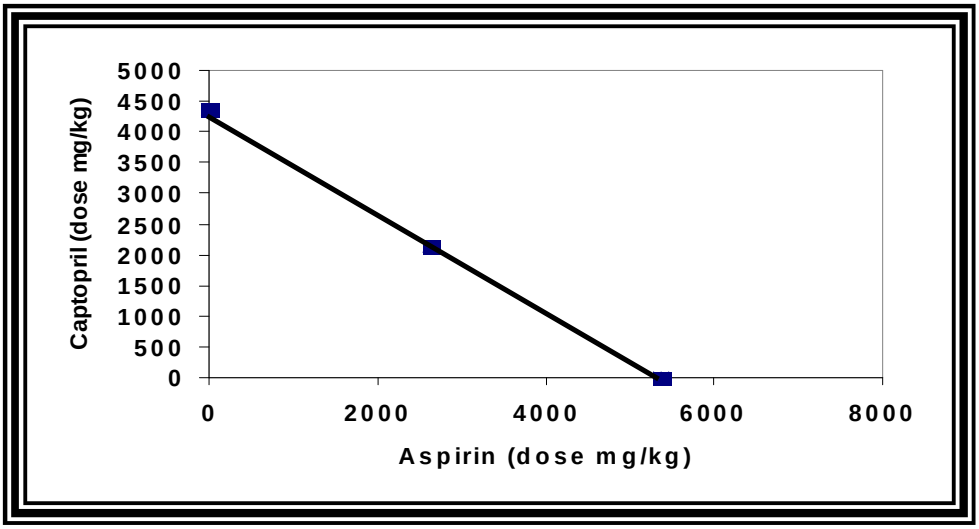

Figure -2 - Isobolographic Analysis For Acute Toxicity Interaction Between Aspirin And Captopri

Chronic Toxicity

Chronic toxicity study showed that LD50 values for captopril and aspirin in T1 & T2 group 3131.5 & 3129.5 mg/kg B.W. respectively. These values further reduced to

1631.5mg/kg for captopril and 2131.5 mg/kg for aspirin in T1& T2 groups after their chronic combination dosing (Table -2-). The same results showed in the T2 groups for aspirin and captopril and their interaction (Table -3).
Isobolographic analysis of two drug LD50 showed that there were an antagonism after chronic toxic exposure. (Figure -3-).

Table - Measurement Of LD50

second Part of serial	+ K : Tests serial				
	O	OO	OOO	OOOO	
XOOO	-0.157	-0.154	-0.154	-0.154	OXXX
XOOX	0.878	-0.861	-0.860	-0.860	OXXO
XOXO	0.701	0.737	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.186	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	-0.305	-0.169	-0.144	-0.142	OOXO
XXXO	1.288	1.500	1.544	1.549	OOOX
XXXX	0.555	0.896	0.985	1.007	OOOO
	X	XX	XXX	XXXX	second Part of serial
	-K : Tests serial				

(Dixon, 1980).

O = survival Animal
X = dead animal

$$LD_{50} = X_f + K_d$$

Table -2- Estimate Of LD50 For Captopril, Aspirin And Their Interaction In T1 Groups After Chronic Toxicity Study

Treatment / Groups	Initial dose mg/kg body weight	Final dose mg/kg body weight	Number in animal	Result after 24 hours	LD50 Mg/kg
Captopril	4000	3500	6	XXOXOX	3131.5
Aspirin	4500	3500	7	XXXOXOX	3129.5
Captopril + Aspirin	2500 + 3000	2000 + 2500	6 + 6	XXOXOX	1631.5 + 2131.5

O = survival animal X= dead animal

Table -3- Estimate Of LD50 For Captopril, Aspirin And Their Interaction In T2Groups After Chronic Toxicity Study

Treatment / Groups	Initial dose mg/kg body weight	Final dose mg/kg body weight	Number in animal	Result after 24 hours	LD50 Mg/kg
Captopril	4000	3500	6	XXOXOX	3131.5
Aspirin	4500	3500	7	XXXOXOX	3129.5
Captopril + Aspirin	2500 + 3000	2000 + 2500	6 + 6	XXOXOX	1631.5 + 2131.5

O = survival animal X= dead animal

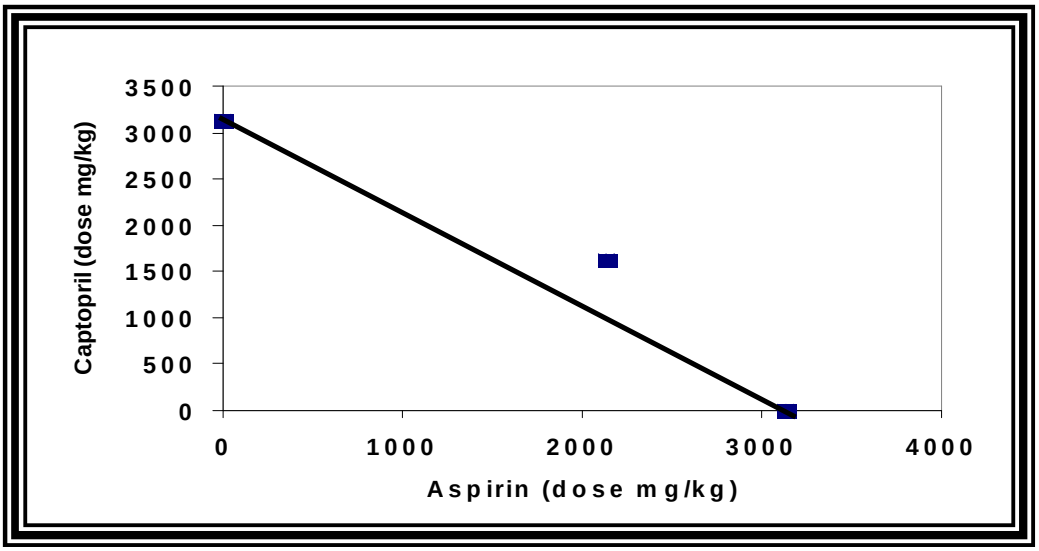

Figure -3 - Isobolographic Analysis For Chronic Toxicity Interaction Between Aspirin And Captopril For T1& T2 Groups

Chronic Index:

Chronic index for aspirin T1&T2 groups and captopril T1&T2 and their combination were calculated according to equation that compare the drug acute LD50 with their one after chronic administration. All Chronic index results for all groups were less than two. No accumulation was reported between the two drugs and their combination after 3 month daily treatment according to the results of chronic index of all treated groups.

$$\text{Chronic index for captopril T1\& T2} = \frac{4370.5 \text{ mg/kg}}{3131.5 \text{ mg/kg}} = 1.48$$

$$\text{Chronic index for aspirin T1\&T2} = \frac{5370.5 \text{ mg/kg}}{3129.5 \text{ mg/kg}} = 1.80$$

$$\text{Chronic index for combination (captopril T1\&T2)} = \frac{2129.5 \text{ mg/kg}}{1631.5 \text{ mg/kg}} = 1.31$$

$$\text{Chronic index for combination (aspirin T1\&T2)} = \frac{2629.5 \text{ mg/kg}}{2131.5 \text{ mg/kg}} = 1.23$$

Discussion

Acute toxicity study

Oral LD50 of aspirin in our acute and chronic study were much higher than the reported one which range between 1500 – 2000 mg/kg (Reingardiene and Lazauskas, 2006) [9] may be because of different rat strain used, while the captopril LD50 result in acute toxicity was nearly similar to the reported one (4450 mg/kg) (Marcel Dekker, 1982) [10].

When captopril and aspirin were given together, the LD50 for either drug was found to be less (captopril 2.1295 g/kg & aspirin 2.6295 g/kg).There are no reported results to compare with these results in our study.

When the results of the drug interaction are characterized in isobolograph diagram, we found that their LD50 intersection point was on the line which mean, there is an additive effect between the two drugs after their administration together may be because the two drugs act mainly on different physiological system (angiotensin system inhibition) and (cyclic prostanoid inhibition) and there is no time to develop tolerance to their toxicity because of acute administration that's why the two drugs LD50 were reduced by the same ratio 50 % after their combination.

Chronic toxicity study

In the this study were found that the results of chronic toxicity were a little bit different from the results of acute toxicity after 3 months treatment.

LD50 values reduce by 28.5 % & 42 % to reach 3.1315 g/kg & 3.1295 g/kg for captopril and aspirin respectively in comparison with that of acute toxicity while their combining intake further reduced after chronic administration to reach 1.6315 g/kg for captopril + 2.1315 g/kg for aspirin.

It seem from LD50 values of both drugs that there is some adaptation or tolerance developed in captopril groups after chronic administration since their LD50 values reduced less than that reported for aspirin groups in comparison with their acute administration ones. This is interesting result taking in consideration that aspirin dosage were within therapeutic ones not as captopril which range a little higher than the therapeutic doses.

When characterized the LD50 of the captopril T1&T2 aspirin T1&T2 and their interaction T1&T2 in chronic toxicity study after 3 months treatment in isobolographic analysis we found that their interaction point is far from the line to the right and that mean, there is antagonism interaction between the two drugs. This finding is in agreement with Moskowitz, (2001)[11] which were reported that Captopril decreased kinin degradation, leading to enhanced production of vasodilator prostaglandins appear to mediate a significant benefit of ACE inhibitor and also leads to an increase in the production of the thromboxane A2. In contrast, aspirin inhibits cylcooxygenase, and thereby suppresses prostaglandin production. Thus, these counteracting effects on prostaglandins may result in antagonism between ACE inhibitor and aspirin therapy in heart failure patients.

Chronic index

Chronic index results for both drug groups pointed that there are no accumulation in all used drugs after their chronic administration may be due to the high excretion of Captopril in a 24-hour period, more than 95 % of the absorbed dose is eliminated in the urine (Duchin, *et al.*, 1988)[12] ,in addition about 75% of the ingested aspirin dose is excreted in the urine from the body, (Lehne, 2001) [13] ,therefore chronic index is less than two that mean there is no accumulation in the body for both drug after their interaction even there is a sign of little toxic effect after chronic administration noticed by decrease LD50 values in all treatment groups in comparison with that of acute toxicity and also the increase in level of clinical enzyme especially ALT, AP as well as BUN with the progress of chronic dosing period positively proportional with the dose.

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Appendix