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KEYWORDS: prodrug , enzyme , cardiovascular disease.

# STUDY OF SOME BIOCHEMICAL VARIABLES OF CARDIOVASCULAR PATIENTS AND EFFECT OF SOME PRODRUGS ON THE ACTIVITY OF SOME ENZYMES IN VIVO AND IN VITRO

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## ABSTRACT:

Several studies have shown the risk factors associated cardiovascular disease (CVD). This research studies some biochemical variables of Cardiovascular patients and effect of some prodrugs on the activity of some enzymes in vivo and in vitro . in this study we estimate some biomarker for cardiovascular patients CK-MB ,LDH ,GPT ,GOT ,Cholesterol ,TG ,HDL ,LDL, VLDL, MDA ,Vitamin C and Vitamin E. Blood samples were collected from 50 cardiovascular patients (25 males and 25 females) and 50 healthy control (25 males and 25 females) from Tikrit hospital, Tikrit city Iraq. Ages of the involved subject range were from 33 to 65 years. Our result show a highly significant increase ($p < 0.005$) in the serum levels of CK-MB , LDH , cholesterol, triglyceride , HDL-cholesterol , GOT ,GPT, MDA in comparison with the control group and significant decrease in Alb ,HDL-Cholesterol ,Vitamin C and vitamin E in comparison with the control . No significant differences between gender (males and females) and no significant differences between ages. Inhibitory effect was study of some prodrugs on CK-MB and LDH activities in serum . The results revealed that the prodrugs have an inhibition effect on the CK-MB and LDH activities with higher inhibition range. The inhibition increased with the increasing concentration of prodrugs while the type of inhibitor showed noncompetitive inhibition. This study was conducted (in vivo) for Ascorbic acid prodrug )Ascorbic acid –Indomethacine( (1) and Glucose prodrug (glucose – Ibuprofe) (2). The concentration of Ibuprofen and Indomethacine was measured in serum after (2, 3, 4, 6, 8, 10) hours of giving a dose to the animal. The results showed that the concentration of Ibuprofen and Indomethacine were the highest after six hours of giving the animal dose. Biochemical study showed inhibition in enzyme activity for prodrugs1 and 2.

### Introduction:

Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels.[2] Cardiovascular disease includes coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack).[2] Other CVDs include stroke, heart failure, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, thromboembolic disease, and venous thrombosis. [3] . The underlying mechanisms vary depending on the disease in question. Coronary artery disease, stroke, and peripheral artery disease involve atherosclerosis. This may be caused by high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, and excessive alcohol consumption, among others.[2] High blood pressure results in 13% of CVD deaths, while tobacco results in 9%, diabetes 6%, lack of exercise 6% and obesity 5%.[2] Rheumatic heart disease may follow untreated strep throat. . It is estimated that 90% of CVD is preventable.[5] Prevention of atherosclerosis involves improving risk factors through: healthy eating, exercise, avoidance of tobacco smoke and limiting alcohol intake. Treating risk factors, such as high blood pressure, blood lipids and diabetes is also beneficial.[2] Treating people who have strep throat with antibiotics can decrease the risk of rheumatic heart disease.[6] The effect of the use of aspirin in people who are otherwise healthy is of unclear benefit.[7][8] .Cardiovascular diseases are the leading cause of death globally. This is true in all areas of the world except Africa.[2] Together they resulted in 17.9 million deaths (32.1%) in 2015, up from 12.3 million (25.8%) in 1990.[4][3] Deaths, at a given age, from CVD are more common and have been increasing in much of the developing world, while rates have declined in most of the developed world since the 1970s.[9][10] Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% of CVD deaths in females. Most cardiovascular disease affects older adults. In the United States 11% of people between 20 and 40 have CVD, while 37% between 40 and 60, 71% of people between 60 and 80, and 85% of people over 80 have CVD.[1] The average age of death from coronary artery disease in the developed world is around 80 while it is around 68 in the developing world.[9] Disease onset is typically seven to ten years earlier in men as compared to women.[11] . There are many risk factors for heart diseases: age, gender, tobacco use, physical inactivity, excessive alcohol consumption, unhealthy diet, obesity, genetic predisposition and family history of cardiovascular disease, raised blood pressure (hypertension), raised blood sugar (diabetes mellitus), raised blood cholesterol (hyperlipidemia), psychosocial factors, poverty and low educational status, and air pollution.[13][14][15][16] While the individual contribution of each risk factor varies between different communities or ethnic groups the overall contribution of these risk factors is very consistent.[17] Some of these risk factors, such as age, gender or family history/genetic predisposition, are immutable; however, many important cardiovascular risk factors are modifiable by lifestyle change, social change, drug treatment (for example prevention of hypertension, hyperlipidemia, and diabetes).[18] People with obesity are at increased risk of atherosclerosis of the coronary arteries.[19] . In the broadest context, biological markers, or biomarkers, are molecules that characterize a biological system or process. In the setting of cardiovascular disease, a number of biomarkers have become an integral part of diagnostic and risk stratification strategies (20) . The most important biomarker was CK-MB , LDH, level of Cholesterol , TG, HDL,LDL, VLDL and other. Creatine kinase (CK) (EC 2.7.3.2), also known as creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme which plays an essential role in the energy metabolism of organisms since it is involved in the synthesis and use of energy-providing molecules[21]

The development of prodrugs — chemically modified versions of the pharmacologically active agent that must undergo a transformation in vivo to release the active drug is now well established as a strategy to improve the physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically potent compounds, and thereby increase the bioavailability and usefulness of a potential drug. For example, prodrugs provide possibilities to overcome various barriers to drug formulation and delivery such as poor aqueous solubility, chemical instability, insufficient oral absorption, rapid [22-24]

### Material and methods:

### Organic study :

Ascorbic acid prodrug (Ascorbic acid –Indomethacine) (1) and

Glucose prodrug (glucose – Ibuprofe) (2) were synthesized and identified according to the literature [24,25]

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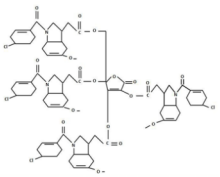
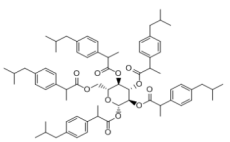


(1)



(2)

### Biochemical study:

This study has two parts:

### 1- In vitro study:

### Patients:

Blood sample was collected from 50 Cardiovascular (CVD) patients and 50 healthy controls (25 males and 25 females) from Tikrit hospital, Tikrit city/ Iraq. Age of the participants was between 33 to 65 years. Ten-milliliter disposable plastic syringes were used to draw venous blood from each patient and control and then the samples were left for 20-30 minutes at 37oC. The serum was used for the analysis after separation by centrifugation. In the present study, LDH and creatine kinase –MB , cholesterol, triglyceride , HDL-cholesterol , GOT ,GPT , AChE and Alb. were estimated using Creatine Kinase-MB , LDH , cholesterol, triglyceride , HDL-cholesterol , GOT ,GPT , AChE and Alb. kits form biolabo company (France) Colorimetric method [27,28] . MDA ,Vitamin C and vitamin E were estimated according to the literature [29,30]

### Effect of the new compounds (1)and (2) on the CK-MB activities in serum:

The effect of the new compounds was calculated at different concentrations (0.5gm/25ml – 0. $5X10^{-7}$gm/25ml). Difference concentrations of the compounds were prepared by serial dilution in DMSO from the stock solution (0.5gm/25ml). The substrate concentration was fixed.

The measurement of enzyme activity was determined by adding 1ml of the compound solution to the substrate buffer.

### Type of inhibition :

An inhibition type study was done at different concentrations of the substrate (0.5- 5 mM) with fixed concentrations of the tested compounds 1 and 2 (0.005 g/25 ml). The enzyme activity was measured in the presence and absence of inhibitor at the same conditions. Linweaver – Burk plot was applied to obtain 1- Km 3- Vmaxand type of inhibition.

### 2- In vivo study:

Twenty five English Angora rabbits weighing (2.5 ± 0.5) Kg were fasted for 48h. Prior to prodrugs administration, the rabbits had free access to drinking water. Each single rabbit was fed 50mg/Kg of tested prodrugs 1and 2. The prodrugs were introduced by a piece of cucumber contaminated with a tested dose. The blood samples were collected by a heart attack method (blood withdrawal from the heart) before and after the prodrugs administration at different intervals of time 0, 2, 3, 4, 6, 8, and 10 hours. They were left at room temperature for half an hour then the serum was separated by centrifugation for 15 minutes at 3500 rpm and stored at -20oC until analysis was performed. The concentration of Ibuprofen and Indomethacine was followed in the wavelength 263.8 and 298 nm respectively (31). In this study, ALP, GOT, GP, ChE, Alb, UA, and CK-MB were estimated using ALP, GOT, GPT, ChE, Alb, UA, and CK-MB LDH , cholesterol, triglyceride , HDL-cholesterol kits form the biolabo company colorimetric method. MDA ,Vitamin C and vitamin E were estimated according to the literature .

## Results and discussion:

In vitro study: The mean (±SD) of creatine kinase (CK-MB) and LDH activity in the serum of control group (healthy individuals) and patients with serum is illustrated in table (1).

**Table (1): Mean ±SD of CK-MB U/L and LDH activity in serum of control group (healthy individuals) and patients**

| | Control | Male | Female | Age (33-50 yrs.) | Age (51-65 yrs.) |
|---|---|---|---|---|---|
| CK-MB U/L | 110.43±14.21 | 253.46±31.66 | 268.56±33.76 | 260.66±34.16 | 263.56±32.42 |
| LDH U/L | 77.41±11.23 | 269.11±35.21 | 280±37.54 | 271±36.64 | 275±36.76 |
| Alb mg/dl | 4.1±1.10 | 2.89±1.05 | 3.1±1.07 | 2.78±1.06 | 3.0±1.08 |
| UA mg/dl | 4.1±1.71 | 6.74±1.21 | 7.24±1.23 | 6.58±1.30 | 6.87±1.31 |
| Cholesterol mg/dl | 125±22.41 | 206±25.11 | 210±27.12 | 222±29.02 | 217±28.32 |
| Cholesterol-HDL mg/dl | 44.76±5.21 | 28.63±2.77 | 30.81±2.98 | 29.56±2.62 | 31.24±3.01 |
| Cholesterol-LDL mg/dl | 101±11.40 | 167±21.36 | 174±22.31 | 171±21.38 | 170±20.22 |
| Cholesterol-VLDL mg/dl | 24.10±2.20 | 39.84±2.50 | 41.54±2.61 | 40.41±2.58 | 42.03±2.76 |
| TG mg/dl | 101±24.51 | 215±26.57 | 226±27.31 | 221±27.11 | 219±26.46 |
| Vitamin C µmol/L | 29.2±2.44 | 17.96±1.86 | 19.70±1.96 | 18.34±1.91 | 19.61±1.94 |
| Vitamin E µmol/L | 20.88±1.78 | 15.85±1.43 | 16.65±1.57 | 15.57±1.88 | 16.21±1.59 |
| MDA mg/dl | 0.871±0.743 | 1.75±0.74 | 1.87±0.75 | 1.78±0.74 | 1.81±0.75 |
| GPT | 34.68±4.62 | 71.34±10.62 | 74.67±11.67 | 72.76±10.91 | 75.21±12.11 |
| GOT | 29.87±3.85 | 67±9.28 | 72.27±10.54 | 70.51±11.17 | 70.87±10.43 |

There is a highly significant increase ($p < 0.005$) in the serum levels of CK-MB , LDH , cholesterol, triglyceride , HDL-cholesterol , GOT ,GPT, MDA in comparison with the control group and significant decrease in Alb ,HDL-Cholesterol ,Vitamin C and vitamin E in comparison with the control . On the contrary, no significant differences between gender (males and females) and no significant differences between age . Our result agree with other studies. The cardio specific isoenzyme of CK-MB and LDH is increased in heart disease. It is noteworthy that our results are consistent with many studies like Damman P et al. (2011) which showed an increase in the level of CK-MB in AMI and acute coronary syndromes (32), while CK-MB as a cardiac marker depends on its relatively high concentration in heart muscle (> 20 %) in comparison with typical skeletal muscle (1 – 2 %). There is an evidence that higher concentrations of CK-MB in the heart may lead to ischemic stress. A number of studies have been shown that the concentration of CK-MB is higher in ventricular myocardial tissue in animal models of hypertrophy or ischemia and in humans with a variety of cardiac conditions in comparison with controls or young individuals without the cardiac disease. In human myocardial biopsy material, concentrations of CK-MB have been reported to be 100-times greater in hearts from patients with aortic stenosis, coronary artery disease, and coronary artery disease with left ventricular hypertrophy compared to patients without such

findings (33). Moreover, increased CK-MB activity was found in conditions such as progressive muscular dystrophy, toxic myopathy, severe poisoning, polymyositis, and severe scleroderma. Therefore, the CK-MB alone is not a good practical way for the diagnosis of AMI or heart disease (34,35). In addition to, CK-MB was elevated due to clearance abnormalities in renal failure or hypothyroidism. Usually, CK-MB becomes elevated in the circulation for 3 – 6h after symptom onset in MI, and remains elevated for 24–36 h. LDH increases in people with heart disease. The results of this study are consistent with many studies such as Manish Bhardwaj et al. (36) Ch Sankeerthi et al. (37), Aharon Erez MD et al. (38) and Srikrishna R et al [39]. They found increased levels of cardiac enzymes like Creatine kinase (CK), Creatine kinase isoenzyme MB (CKMB), LDH in comparison with controls. Transaminases, lipid profile like Triacylglycerol level, Cholesterol, LDL and Homocysteine does not show any significant difference in comparison with controls in selected Myocardial infarction (MI) patients. There are indicators to a strong interconnection between the cardiac enzymes, lipid profile and homocysteine level with the AMI, Therefore, implying possible risk factors for MI. Lactate Dehydrogenase was significantly raised in the Coronary Artery disease patients as compared to normal healthy individuals Our results showed a decrease in the serum levels albumin in heart disease , and this agrees with other studies Nehal Rachit Shah (40) , Sathi S., et,al(41) and Dick de Zeeuw etal (42).

There is a highly significant increase ($p<0.005$) in serum levels of cholesterol, triglyceride, LDL and VLDL, and a significant decrease ($p<0.05$) in serum levels of HDL when compared with the control group. Our results agree with schwaiger et al.(2006) who studied the cardiovascular events and lipid abnormalities in CKD patients. In their study the lipid abnormalities were elevated serum total cholesterol, HDL, LDL and triglyceride levels(43) . and disagree with Mannangi N et al.(2014) who demonstrated significant decreased HDL, but no change in total cholesterol, LDL(44) There is a highly significant decrease ($p<0.005$) in serum levels of vitamin C and vitamin E and a significant increase ($p<0.05$) in serum levels of MDA when compared with the control group. Our results agree with Dennis, V & parke, D. (1994)(45) and Velasco etal[46] .

**Effect of compounds (1) and (2) on CK-MB and LDH activity in serum patients:**

The effect of compound (1) and (2) and the mean (±SD) of creatine kinase (CK-MB) and LDH activity in serum is illustrated in table (2).

**Table (2): The effect of compounds (1) and (2) on CK-MB and LDH activity in serum patients.**

| Compounds 1 | CK-MB Activity U/L | LDH Activity U/L |
|---|---|---|
| Nil | 268.56±33.76. | 280.66±37.54 |
| 0.5mg/25ml | 126.11±13.19 | 134.17±12.22 |
| $0.5x10^{-1}$mg/25ml | 135.23±14.98 | 151.23±24.60 |
| $0.5x10^{-2}$mg/25ml | 147.11±19.44 | 166.33±25.36 |
| $0.5x10^{-3}$mg/25ml | 166.43±23.51 | 182.64±27.18 |
| $0.5x10^{-4}$mg/25ml | 197.36±26.18 | 208.43±32.55 |
| $0.5x10^{-5}$mg/25ml | 234.31±31.22 | 241.29.±34.15 |
| $0.5x10^{-6}$mg/25ml | 250.11±33.21 | 271.52±36.65 |
| Compound 2 | CK-MB Activity U/L | LDH Activity U/L |
| Nil | 268.56±33.76. | 280±37.54 |
| 0.5mg/25ml | 131.22±14.20 | 147.21±13.32 |
| $0.5x10^{-1}$mg/25ml | 145.34±15.31 | 160.32±19.42 |
| $0.5x10^{-2}$mg/25ml | 157.44±19.19 | 176.31±23.17 |
| $0.5x10^{-3}$mg/25ml | 172.13±21.42 | 187.62±24.23 |
| $0.5x10^{-4}$mg/25ml | 205.36±26.18 | 221.44±32.17 |
| $0.5x10^{-5}$mg/25ml | 241.31±32.11 | 252.57.±35.61 |
| $0.5x10^{-6}$mg/25ml | 257.51±33.35 | 274.54±38.71 |

The effect of the new prodrugs on the activity of the CK-MB and LDH outside of the body was tested (in vitro). When the increasing concentration of the new compounds (prodrugs) in comparison with patients, Inhibition of the enzyme activity decreased. The activity of CK-MB and LDH in patients was (268.56 ± 33.76 U/L) and (280 ± 37.54 U/L) respectively. The activity of CK-MB and LDH with the new prodrugs (Inhibitor) was range (126.11 ± 13.19 - 250.11 ± 33.21) U/L, (141.32 ± 15.55 - 262.51 ± 33.35) U/L and (131.22 ± 14.20 - 257.51 ± 33.35) U/L for CK-MB with compounds 1, 2, and 3 respectively and (134.17 ± 12.22 - 271.52 ± 36.65) U/L, (154.21 ± 13.44 - 278.58 ± 38.81) U/L and (147.21 ± 13.32 - 274.54 ± 38.71) U/L for LDH with compounds 1 and 2 respectively. In the concentration 0.5x10-5mg/25ml and 0.5x10-6mg/25ml was found that activity of the enzyme approach to the activity in patients because of the large dilution of the compounds. The effect of compound1 is greater than compound 2 because the compound 1 has more carbonyl groups then compound (2). As a result, these groups effect on the residue amino acid containing the active site of CK-MB and LDH (CK-MB transfer of a functional group from one substance to another. The functional group may be methyl-, acyl-, amino-, phosphate and LDH catalyze oxidation/reduction reactions. Therefore, it leads to transfer of H and O atoms or electrons from one substance to another. Synthesized compounds have functional group (ester groups) and the carbonyl group has dual electronic.

The Linweaver – Burk relation showed that the type of inhibition was non-competitive inhibition (Km value unchanged and the Vmaxi was less than Vmax observed in the absence of inhibitor) Table (3) and figures (1).

**Table (3) Km, Vmax and Vmaxi for CK-MB**

| | Control | With compound 1 | With compound 2 |
|---|---|---|---|
| Km mM | 0.4 | 0.4 | 0.4 |
| Vmax (IU/L) | 83.3 | | |
| Vmaxi(IU/L) | | 66.66 | 47.6 |

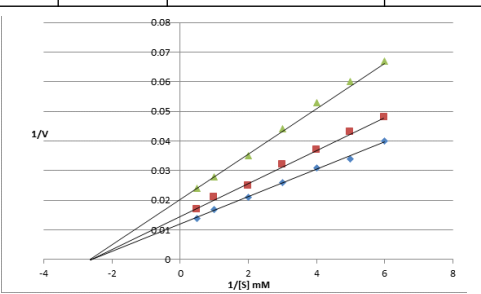



**Fig. (1) linwever-burk plot for CK-MB**

The results are consistent with many studies such as Abi Beaulah G et al. (47), Vishal V.Kshirsagar et al (48), GOMATHI R et al (49) , Yogita Dobhal (50) and Thamolwan Suanarunsawat (51). They found methanolic extract from Croton sparciflorus. As well they studied the cardioprotective activity using Isoproterenol induced myocardial infarction in wistar albino rats. Methanolic extract of plant showed a significant cardioprotective effect by lowering the serum levels of various biochemical parameters such as CPK, LDH, and transaminases in the selected model the results obtained are in turn with histopathological examinations of heart tissue sections and are comparable with the standard cardioprotective drug. Moreover, the results suggest that the biologically active phytoconstituents such as flavonoids, glycosides, alkaloids present in the methanolic extract from plant. The levels of LDH and CK-MB in the differ experimental groups, The ISO-induced rats showed a significant ($p < 0.05$) increase in the level of LDH and CK-MB in comparison with the normal rats. However, the group V and VI showed a decrease in the level of cardiac marker enzymes in comparison with ISO-induced rats. The administration of EMsS extract alone treated the group. The methanolic extract from Carica

papaya leaves significantly prevented the myocardial infarct size and markedly reduced the LDH and CK-MB release in coronary effluent at the dose level of 100mg/kg body weight in comparison with that of the standard ramipril (2.5mg/kg body weight).

**In vivo study:**

In this study 50mg/Kg of the compounds (1) and (2) were used (oral administration). The concentration of Ibuprofen after (2h) was ((2.2X10-6), (3h) (9.4X10-6), (4h) (3.1X10-5), (6h) (6.7X10-5), (8h) (1.4X10-5), (10 h) (3.7X10-6 M) while concentration of Indomethacine after (2h) was (1.1X10-6, (3h) (3.7X10-6), (4h) (1.5X10-5), (6h) (4.6X10-5), (8h) (1.1X10-5), (10h) (2.1X10-6 M.Table (4).

The results showed that the concentrations of Ibuprofen and Indomethacine were low in the first hour and then increased with time until 6 hours (the maximum concentration of Ibuprofen and Indomethacine). After (8 - 10h), the concentration of aspirin was decreased. On the other hand, after 10 hours the concentration of Ibuprofen and Indomethacine became equal to the concentration of Ibuprofen and Indomethacine during two hours. Hydrolysis normally accomplished by esterase enzyme present in the serum and other tissues capable of hydrolyzing a wide variety of ester linkages like (Ester hydrolase, Lipase, Cholesterol esterase, Acetyl cholinesterase, Carboxypeptidase) (52).

**Table (4) the concentration of Ibuprofen and Indomethacine rabbit blood serum (In vivo)**

| Time | Concentration of Ibuprofen M | Concentration of Indomethacine M |
|---|---|---|
| 0 | 0 | 0 |
| 2 | 2.2X10-6 | 1.1X10-6 |
| 3 | 9.4X10-6 | 3.7X10-6 |
| 4 | 3.1X10-5 | 1.5X10-5 |
| 6 | 6.7X10-5 | 4.6X10-5 |
| 8 | 1.4X10-5 | 1.1X10-5 |
| 10 | 3.7X10-6 | 2.1X10-6 |

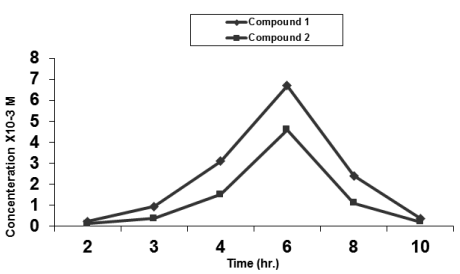



figure (1) concentration of Ibuprofen and Indomethacine relesed

Jun-ichi Yokoe et al (2003) (52) referred to Plasma concentration–time profiles of both SASA and 5-ASA after oral administration of SASA. They were predicted very well by introducing a factor for the first-pass elimination of 5-ASA into the GITA model. In this work, the simulation study using the parameters obtained. The present work showed about 94.7 % of SASA reaches the cecum, where 5-ASA is regenerated very rapidly and 76.0 % of 5-ASA is absorbed. Furthermore, the bioavailability of 5-ASA was estimated about 0.330 because of the first-pass elimination through both cecum and liver. In conclusion, the absorption behaviors of a prodrug and its regenerated parent drug was predicted very well and clarified successfully using the GITA model. Joachim Brouwers et al (53) referred to the development of phosphate ester prodrugs is an interesting approach to increase intestinal absorption of poorly water-soluble drugs. Absorption of a drug from its phosphate ester prodrug is based on intestinal dephosphorylation of the prodrug which may result in intraluminal supersaturation of the parent drug. Hanna Kumpulainen(54) et al referred to (3) greatly enhanced the aqueous solubility of propofol (solubility over 10 mg/mL) and the stability in buffer solution (t1/2 = 5.2 ± 0.2 days at pH 7.4, r.t.) was sufficient for i.v. administration. The enzymatic hydrolysis of (3) to propofol was extremely rapid in vitro (t1/2 =21 ± 3 s). Moreover, (3) was readily converted to propofol in vivo in rats. During bioconversion, (3) has the capability to releases acetaldehyde. Acetaldehyde is a less toxic compound than the formaldehyde, which releases from the phosphonooxymethyl prodrug of propofol (Aquavan). Currently, it is undergoing clinical trials. The maximum plasma concentration of propofol was 3.0 ± 0.2g/m. It was reached within 2.1 ± 0.8 min after the i.v. administration of (3). The present study indicates to ethyl dioxy phosphate represents a potentially useful water-soluble prodrug structure suitable for i.v. administration.Qiong Xie et al(55). The prodrug (Z)-3-[2-(propionyloxy) phenyl]-2-propenoic estershowed a 4-fold increase in oral bioavailability over the parent drug meptazinol in rats. Arik Dahan et al (56) following oral or intra-colon DP-155 administration, free indomethacin was liberated along the intestine and absorbed into the systemic circulation, resulting in a controlled release profile of indomethacin in the plasma. The shorter linker caused a 20-fold decrease in the subsequent indomethacin absorption. DP-155 in-vitro degradation by PLA2 was over 60%, while shorter linkers were profoundly less degradable. Conclusions: DP-155 caused a continuous input of free indomethacin into the plasma following the degradation by PLA2 in the gut lumen. Since the rate of drug release is not formulation dependent, the prodrug can be compounded even in a liquid dosage form. The phospholipid–drug conjugate is thus a potential novel mechanism for oral controlled release of drugs In the presentwork, we are studying ALP, GOT, GPT, ChE, Alb, UA and CK-MB which were measured in vivo. It was found that the activity of enzymes was reduced (inhibition the enzyme activity) as showed in table (5)

**Table (5) the effect of compounds 1 and 2 on ALP, GOT, GPT, ChE, Alb, UA and CK-MB activity in vivo**

| | | Activity | % Inhibition | Activity | % Inhibition |
|---|---|---|---|---|---|
| | | Compound 1 | | Compound 2 | |
| ALP | Control | 78.44±2.2 U/L | 14.903 | 78.44±2.2 U/L | 21.659 |
| | Test | 66.75±3.4 U/L | | 61.45±2.4U /L | |
| GOT | Control | 51.65±1.2 U/L | 17.618 | 51.65±1.2 U/L | 23.814 |
| | Test | 42.55±4.1 U/L | | 39.35±4.4U /L | |
| GPT | Control | 63.37±4.0 U/L | 20.640 | 63.37±4.0U /L | 23.418 |
| | Test | 50.29±3.3 U/L | | 48.53±3.1U /L | |
| ChE | Control | 409.25±3.3 U/L | 5.431 | 409.25±3.3 U/L | 7.609 |
| | Test | 387.02±2.3 U/L | | 378.11±2.1 U/L | |
| Alb | Control | 4.10±0.8 gm/dl | 12.195 | 4.10±0.8 gm/dl | 15.853 |
| | Test | 3.60±0.6 gm/dl | | 3.45±0.5 gm/dl | |
| UA | Control | 4.66±1.1 mg/dl | 11.587 | 4.66±1.1 mg/dl | 19.742 |
| | Test | 4.12±1.0 mg/dl | | 3.74±1.2 mg/dl | |
| CK-MB | Control | 98.78±6.5 U/L | 11.156 | 98.78±6.5 U/L | 17.452 |
| | Test | 87.76±5.1 U/L | | 81.54±4.5U /L | |

The activity of the enzyme was measured after and before Dosing of the animals tested. The result indicated that the inhibition ranges of GOT, GPT, ChE, Alb, UA and CK-MB (14.903, 17.618, 20.640,5.431, 12.195, 11.587, 11.156) % compound (1) and (21.659, 23.814, 23.418, 7.609, 15.853, 19.742, 17.452)% for compound (2) respectively.

Nonsteroidal anti-inflammatory drugs such as ibuprofen and Indomethacine work by inhibiting the COX enzymes, which convert arachidonic acid to prostaglandin H2 (PGH2). PGH2, in turn, is converted by enzymes to several prostaglandins (which are mediators of pain, inflammation, and fever) and to thromboxane A2 (which stimulates platelet aggregation, leading to the formation of blood clots).The exact mechanism of ibuprofen activity is unknown. Ibuprofen is a nonselective inhibitor of cyclooxygenase, an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway. Its pharmacological effects are believed to be due to inhibition of cyclooxygenase-2 (COX-2) which decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever, and swelling. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation. Inhibition of COX-1 is thought to cause some of the side effects of ibuprofen including gastrointestinal ulceration. Ibuprofen is administered as a racemic mixture. The R-enantiomer undergoes extensive interconversion to the S-enantiomer in vivo. The S-enantiomer is believed to be the more pharmacologically active enantiomer.[57] . Similar aspirin and indometacin, ibuprofen is a nonselective COX inhibitor. It inhibits two isoforms of cyclooxygenase, COX-1, and COX-2. The analgesic, antipyretic, and anti-inflammatory activity of NSAIDs appear to operate mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on the gastrointestinal tract.(58) However, the role of the individual COX isoforms in the analgesic, anti-inflammatory, and gastric damage effects of NSAIDs is uncertain and different compounds cause different degrees of analgesia and gastric damage.[59]

Holt S et al refer to a series of analogues of ibuprofen and indomethacin that have been investigated with respect to their ability to inhibit fatty acid amide hydrolase, the enzyme responsible for the hydrolysis of the endogenous cannabinoid anandamide. Over the fourteen compounds were tested. The 6-methyl-pyridin-2-yl analogue of ibuprofen ("ibu-am5") was selected for further study. This compound inhibited rat brain anandamide hydrolysis in a non-competitive manner, with IC50 values of 2.5 and 4.7 microM being found at pH 6 and 8, respectively. By comparison, the IC50 values for ibuprofen were 130 and 750 microM at pH 6 and 8, respectively. There was no measurable N-acylethanolamine hydrolyzing acid amidase activity in rat brain membrane preparations. In intact C6 glioma cells, ibu-am5 inhibited the hydrolysis of anandamide with an IC50 value of 1.2 microM. There was little difference in the potencies of ibu-am5 and ibuprofen towards cyclooxygenase-1 and -2 enzymes, and neither compound inhibited the activity of monoacylglycerol lipase. Ibu-am5 inhibited the binding of [3H]-CP55,940 to rat brain CB1 and human CB2 cannabinoid receptors more potently than ibuprofen, but the increase in potency was less than the corresponding increase in potency seen for inhibition of FAAH activity. It is concluded that ibu-am5 is an analogue of ibuprofen with a greater potency towards fatty acid amide hydrolase but with a similar cyclooxygenase inhibitory profile, and may be useful for the study of the therapeutic potential of combined fatty acid amide hydrolase-cyclooxygenase inhibitors[60].

Mayeux PR et al referred to ibuprofen may inhibit thromboxane (TX) A2 synthase activity and inhibiting cyclooxygenase activity. Microsomal fractions were isolated from the cat lung containing cyclooxygenase as well as prostacyclin (PGI2) synthase, TX synthase, and a GSH-dependent prostaglandin (PG) E2 isomerase activities. When [1-14C] PG endoperoxide H2 (PGH2) was used as a substrate, ibuprofen, indomethacin, and meclofenamate exhibited differential effects on terminal enzyme activities. Ibuprofen at concentrations up to 1mM, had no effect on the activities of PGI2 synthase, TXA2 synthase of GSH-dependent PGE2 isomerase. Whereas indomethacin at 5 X 10(-4) M and 10(-3) M selectively inhibited PGI2 synthase activity. Meclofenamate at 5 X 10(-4) M and 10(-3) M selectively inhibited TXA2 synthase activity. However, at concentrations of 5 X 10(-3) M, this selectivity was not observed. Idomethacin and meclofenamate decrease lead to the formation of both 6-keto-PGF1 alpha and TXB2. These data indicate that the choice of NSAID and the concentration used may specifically alter PGH2 metabolism. This action may affect the physiologic consequences of the exchange of PGH2 between cells. The data further indicate that indomethacin has potential using as a tool to specifically attenuate PGI2 synthase activity in vitro[61].

Yang EM referred to 26 infants received indomethacin and 22 infants received ibuprofen were studied. The overall rate of ductal closure was similar between the two treatments: (a) it occurred in 23 of 26 infants (88.5%) treated with indomethacin and (b) in 18 of 22 of infants (81.8%) treated with ibuprofen (p=0.40). Interestingly, the rate of surgical ligation (11.5% versus 18.2%; p=0.40) did not differ significantly between the two treatment groups. Moreover, there was no significant difference was found in post-treatment serum creatinine concentrations between the two groups and no significant differences in additional side effects or complications. Oral ibuprofen is as efficacious as intravenous indomethacin for the treatment of PDA. There were no differences between the two drugs with respect to safety. Oral ibuprofen could be used as an alternative agent for the treatment of PDA in ELBW infants[62].

Authors' contributions

rugs with respect to safety. Oral ibuprofen could be used as an alternative agent for the treatment of PDA in ELBW infants[62].

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Competing interests

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Availability of data and materials

All data generated or analyzed during this study are included in this articles.

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