

Quick Pharmaceutical steps for preliminary evaluation of a compound as a possible oral prodrug

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الخلاصة:

مقدمة الدواء التوافقية هي مشاركة عقاران مختلفان متصلان مع بعضهما البعض مكونا جزيئه فريدة من نوعها لإعطاء تأثيرتازري. مقدمة الدواء التوافقية المكونة من الأسبرين والبراسيتامول تم تحضيره كموديل لمقدمة دواء فموية و تم تشيخه كيميائيا وتقييمه صيدلانيا بالدراسات التالية: دراسة الإذابة ودراسة سرعة التحلل في محاليل الدرء ذات أس هيدروجيني من 1 الى 9 مشابهها الى الاس الهيدروجيني للجهاز الهضمي و دراسة التحلل باستعمال البلازما البشرية. اظهرت النتائج ان استقرارية المركب فى الجهاز الهضمي اكبر من سرعة عبوره فى المعدة و الامعاء و هذا سيساعد على امتصاص الدواء قبل تحلله و كذلك يتحلل المركب فى الدم بسرعة اعلى بكثير من سرعة خروجه من الجسم وبالتالي تعتبر هذه الطريقة معتمدة لغرض التقييم الاولى لاي مركب يحضر على انه مقدمة دواء و فى حاله اجتيازه هذه التحاليل بنجاح و حسب التقديرات المقترحه يمكن بعده اجراء دراسات صيدلانية موسعة لاثبات امكانية اعتبار المركب المحضر كمقدمة دواء ناجح للاستعمال الفموى.

Abstract:

A mutual prodrug is the association in a unique molecule of two, usually synergetic drugs attached to each other. Mutual prodrug of aspirin with paracetamol as a model oral prodrug was prepared and identified and many pharmaceutical steps were applied including: solubility studies, hydrolysis of the prodrug in different pH from 1 to 9 to simulate the pH of the GIT and hydrolysis of the prodrug by human plasma. The results showed that the stability of the compound in the GIT should be more than its transient time in the GIT in order to be absorbed before it is hydrolyzed, and the compound should be hydrolyzed after it is absorbed from the GIT with in suitable time in comparison to its elimination half life. These studies can be considered as quick pharmaceutical studies for preliminary evaluation of any suggested prodrug, if the compound passes

these steps successfully then further pharmaceutical studies is suggested for the final recommendation.

Introduction:

A therapeutically significant drug may have limited utilization in clinical practice because of poor organoleptic properties, poor bioavailability, and short duration of action, nonspecificity, incomplete absorption, poor aqueous solubility, high first-pass metabolism or other adverse effects. There is great emphasis on research to discover methods aimed at improving their therapeutic efficacy by minimizing or eliminating these undesirable properties. Sometimes, an adequate pharmaceutical formulation can overcome these drawbacks but often the galenic formulation is inoperrant and a chemical modification to convert an interesting active molecule into acceptable drug is necessary. The chemical formulation process, whose objective is to convert an interesting active molecule into a clinically acceptable drug, often involves the so-called Prodrug design ^[1]. Mutual prodrug is a type of carrier-linked prodrug, where the carrier used is another biologically active drug instead of some inert molecule. A mutual prodrug consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and *vice versa*. Mutual prodrug design is really not different from the general drug discovery process, in which a unique substance is observed to have desirable pharmacological effects, and studies of its properties lead to the design of better drugs. It is a very fruitful area of research, and its introduction in human therapy has given successful results in improving the clinical and therapeutic effectiveness of drugs suffering from some undesirable properties that otherwise hinder their clinical usefulness ^[2,3]. Aspirin is a well known analgesic antipyretic drug. It can cause gastrointestinal damage, including lesion, peptic ulcers and bleeding. These side effects are attributed to the presence of free COOH group therefore blocking this group by synthesizing functional derivatives of carboxylic acid may reduce these side effects ^[4]. In the present work pharmaceutical investigations had been carried out on a mutual prodrug of aspirin and paracetamol which is synthesized (to be used as a model prodrug) in order to present some quick pharmaceutical steps that can be helpful in preliminary evaluation of a product for its potential application as a prodrug .

Materials and Methods:

Synthesis of the prodrug:

Aspirin (0.15 mol) changed to its corresponding acid chloride then paracetamol (0.15 mol) added into a round-bottomed flask, add a little

amount of water until all the powder dissolve. Add one or two boiling stones. Assemble a reflux apparatus, and heat the reaction mixture at reflux for 2hours. Remove the heat source and let the mixture cool at room temperature. Pour the cooled mixture into a separatory funnel and add carefully 50 ml of cold distilled water. Rinse the reaction flask with 5 ml cold distilled water and also add to separatory funnel. Pour 10 ml of 0.5M sodium bicarbonate solution into the separatory funnel. Stir the two phases with stirring rod until the evolution of CO₂ gas nearly stop. Remove the lower aqueous layer and repeat the extraction with another 25 ml NaHCO₃, wash the organic layer for the last time with 20 ml NaHCO₃. Remove the aqueous layer again and pour the ester into a dry 50 ml flask. Dry the product with anhydrous calcium chloride and allow it to stand over drying agent for 30-40 min. ^[5].

Identification of the product:

- 1- Melting point:** The melting point of the product is 155°C.
- 2- TLC:** By using Aluminum sheets with SG thickness 0.25mm and solvent system Toluene: n-Propanol: formic acid (5:4:1) and visualization is carried out using Iodine vapor ^[6].
- 3- UV scanning:** UV scan for the prodrug in buffers and methanol were carried out using UV-165-OPC-Shimadzu.
- 4- IR:** IR spectrum was carried out by KBr disc using FTIR-8400 S-Shimadzu.

Preliminary evaluation of the product as a possible prodrug:

1- Solubility determination of the product:

Excess amount of product was added to buffer and methanol, mixed and left in a shaking water bath at 25°C for 30 min. Then filtered and the absorbance of the filtrate was measured spectrophotometrically at 290 nm. The absorbance was converted to concentration using prepared standard absorbance versus concentration curves.

2- Effect of pH on the stability of the product:

The hydrolysis of the compound is followed up in different pH 1 to 9 using phosphate buffer(0.1M) at 37°C, where 5 ml samples were withdrawn at different intervals and analyzed by U.V at 290 nm, in order to study the effect of different pH (simulating the pH of the G.I.T fluid) ^[7]

3- Hydrolysis of product in human plasma:

Five milliliters of plasma was equilibrated at 37°C in a water bath for 30 min. A stock solution of the prodrug was prepared and 100 µL of this solution were added to the plasma ,then mixed , 100 µL samples were withdrawn at different intervals, the reaction was quenched with 3 ml of 0.01M pH 4.5 phosphate buffer, the resultant mixture was

centrifuged and then the supernatant was analyzed spectrophotometrically at 262 nm and the appearance of aspirin was followed against blank of plasma solution which was treated in the same way^[8].

Result and Discussion:

The product obtained from the esterification of aspirin and paracetamol was identified where the melting point was found to be 155°C and the UV scan yielded peak at a wave length of 290 nm in buffers (different from that of Aspirin and paracetamol) and 308nm in methanol. The IR spectrum showed two carbonyl stretching vibrations at 1681 and 1751 cm^{-1} for two ester groups and at 3331 cm^{-1} for secondary amine. Additional identification was done by thin layer chromatography where the Rf value is 0.15 for product, 0.3 for aspirin and 0.68 for paracetamol. The results obtained were in a good agreement with the reported data^[3].

Solubility in buffer for the prodrug was found to be 11.5 mg / ml and the compound showed to be freely soluble in methanol (1 gm in 4 ml methanol).

The effect of pH on the rate of hydrolysis of the prodrug was investigated in buffer aqueous solution over different range of pH 1 to 9 using 0.1 M phosphate buffer simulating different pH of the GIT content, and the overall hydrolysis showed specific acid – base catalysis for protonated and unprotonated forms of ester in addition to water catalyzed reaction at pH 4- 6 and it is agreed with reported data for similar ester like structure^[9].

The hydrolysis of the prodrug was studied in human plasma at 37 °C to get idea about its behavior in vivo. The prodrug hydrolyzed to aspirin which was detected by following the appearance of aspirin at 262 nm. The result showed that there is increase in concentration of aspirin with time and it follows 1st order kinetic by plotting log concentration versus time curve as shown in fig.1 with regression r^2 0.9464 and the rate of hydrolysis constant equal to 0.0168 min^{-1} with respective half life calculated as 0.693/k equal to 41 min. The extent of catalysis depends on the steric factor of the functional groups in the structure of the prodrug.

From the overall results, the prepared ester showed good stability in the buffer solutions in wide pH range indicating that the ester is not hydrolyzed during its transit time through the stomach and intestine in addition to its solubility which will give enough time for the ester to be absorbed in the GIT and so this approach may enhance the bioavailability of the ester. In addition to that the ester cleaved back to give the parent drugs (aspirin and paracetamol) after it is absorbed by the plasma

enzymes and both of prodrug moieties are not toxic and this ester will not only improve the gastric tolerance of aspirin but also improve its pharmacological profile due to the synergistic action showed by paracetamol .

It is necessary to do such quick pharmaceutical steps to have preliminary investigation about the possible consideration of the prepared compound to be an oral prodrug depending on its stability in different rang of pH , similar to that of stomach and intestine which will allow enough time for the compound to be absorbed in addition to its fast cleavage to give the parent compounds after it is absorbed in plasma in comparison to its biological half life, besides its acceptable solubility which will affect the choice of the suitable oral dosage form in which the compound will be presented. If the prodrug passes these steps successfully then it is recommended to do further investigations including the study of different factors that affect the stability of drug in GIT such as the buffer types and concentrations, ionic strength and pH – rate profile in addition to effect of factors affecting the absorption of the drug from the GIT, and the effect of temperatures on the stability of the prodrug which will determine the expiration date in addition to different other factors to be considred and studying the pharmacology and toxicity of the prodrug.

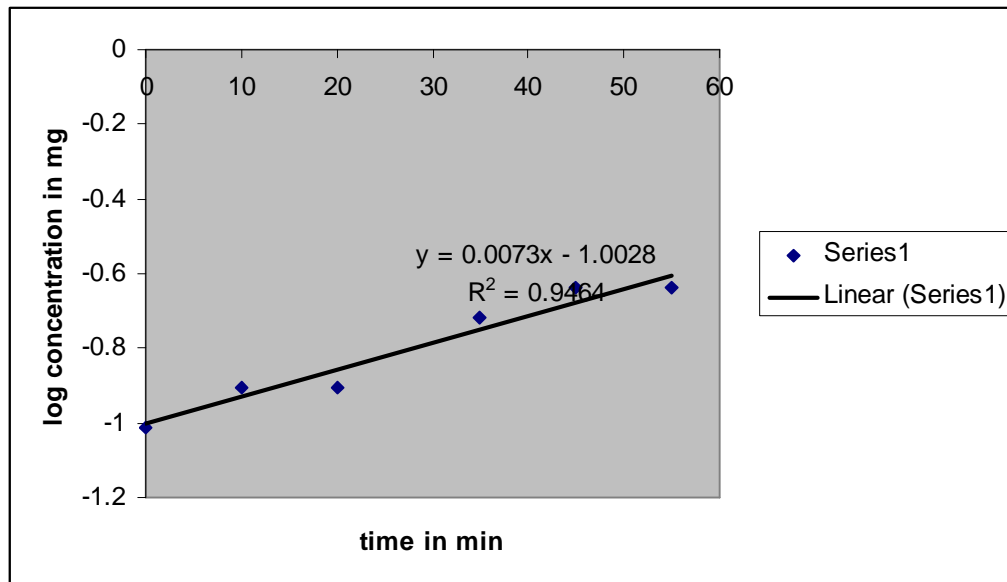


Fig-1: First order plot of the appearance of aspirin by the hydrolysis of the ester in human plasma.

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