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Effect of Ketoprofen on Lactic Dehydrogenase from Human Platelets

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. In different clinical investigations of thrombocytopenia, ketoprofen was found to be the associated cause. Ketoprofen alone or in combination with other therapeutic regimens leads to a decrease in platelet count. Thrombocytopenia due to ketoprofen use can be a threatening condition to the patients who require uncompromised platelet function.
Objectives. In order to establish a mechanism for thrombocytopenia associated with ketoprofen use, the enzyme inhibition effects of ketoprofen on lactic dehydrogenase (LDH) were investigated in this study. LDH is essentially involved in platelet energy production.
Material and Methods. LDH isolated from human platelets was subjected to different concentrations of ketoprofen (250, 500, 750, 1000 and 1500 μg/mL) and pyruvate as a substrate (45, 60 and 90 μM/mL) to gain insight into the enzyme inhibition effects for forward reaction. Oxidation of nicotinamide adenine dinucleotide (NADH) was measured at 340 nm to evaluate enzyme activity. Enzyme inhibition kinetics were studied via Lineweaver Burk plot.
Results. Ketoprofen was found to be a competitive inhibitor of LDH in human platelets. 89% of enzyme activity was inhibited by a 1500 μg/mL concentration of the drug and the enzyme inhibition constant was 882 μg/mL.
Conclusions. The possible main cause of thrombocytopenia due to ketoprofen use is LDH inhibition in platelets, which are essential for platelet energy metabolism. So patients who require uncompromised platelet function and are receiving ketoprofen in their prescription should be monitored for platelet count and blood clotting (**Adv Clin Exp Med 2014, 23, 3, 377–380**).

Key words: ketoprofen, thrombocytopenia, lactic dehydrogenase, enzyme inhibition, pyruvate, kinetics.

Platelet function is vital for both hemostasis and thrombosis, mainly participating in the coagulation cascade for thrombus formation. According to the patient's disease condition, platelet inhibition may be required as in percutaneous catheter intervention, myocardial infarction stent thrombosis and to minimize thrombotic complications to prevent adverse cardiovascular events [1, 2]. However, in some cases platelet function becomes essential e.g. hemorrhagic thrombocytopenia in dengue fever and immune thrombocytopenic purpura [3, 4]. It therefore requires a great deal of care in defining the drugs to be included in the therapeutic regimens for such patients. Ketoprofen is a potent nonsteroidal anti-inflammatory agent (NSAID) from the 2-arylpropionic acid class, efficacious in pain management, used as an analgesic, anti-inflammatory and anti-arthritic agent. NSAIDs from the propionic acid class have found a suitable place in prescriptions dealing with pain management [5]. However ketoprofen and other propionic acid-derived NSAIDs, upon clinical investigation, have shown life-threatening thrombocytopenia and thus are advised to be used with caution, especially in patients in whom compromised platelet function will be a concerning threat [6, 7]. Lactic dehydrogenase (LDH) is pivotal in the energy metabolism of platelets and thus its functionality

is necessary for platelet activation and aggregation [8–10]. The current study is designed to access the inhibitory effects of ketoprofen on LDH, in order to establish a possible explanation for the thrombocytopenia associated with ketoprofen use. Enzyme kinetics were determined via a lineweaver burk plot, along with a type of enzyme inhibition by incubating different concentrations of ketoprofen and pyruvate with nicotinamide adenine dinucleotide (NADH) as a substrate for forward enzyme reaction.

Material and Methods

Freshly donated blood was obtained from the Amna Blood Bank, Multan, Pakistan. Sodium pyruvate, NADH (Merk, Germany), phosphate buffer pH 7.5 (BDH, England) and all other chemicals were purchased from a local vendor and used without any further modification. Ketoprofen powder was gifted by Aventis Pharma, Karachi, Pakistan.

Preparation of LDH from Platelets

For each measurement, 10 mL of venous blood was collected in centrifuge tubes containing 3.8 mg sodium citrate and centrifuged at 1000 g for 15 min at 25°C. Supernatant i.e. platelet rich plasma (3.5 mL) was collected and centrifuged for 15 min at 2000 g and 25°C to obtain platelet pellets. The pellets were washed twice with 5 mL normal saline to make the serum LDH activity zero. The platelets were lysed by homogenization for 10 min in order to have maximum LDH activity.

Determination of Enzyme Inhibition

LDH inhibition was measured spectrophotometrically by mixing a 50 mM phosphate buffer (pH 7.5) in the above-mentioned LDH preparation [10, 11]. Solutions of different stated concentrations of ketoprofen were prepared to evaluate the inhibition effects. Sodium pyruvate (with NADH 0.18 mM) was used as a substrate to determine the inhibition of LDH for forward reaction of LDH [12]. Different concentrations of pyruvate were used to gain insight into the type of enzyme inhibition. A 3 mL buffered reaction mixture, consisting of 20 µL LDH preparation along with different concentrations of pyruvate and ketoprofen, was incubated for 5 min and a decrease in absorbance was measured at 340 nm for NADH oxidation. The results are given as a mean of the triplet. The enzyme activity was expressed as units per liter (U/L). Enzyme kinetics were interpreted via Lineweaver–Burk plot for nonlinear expression [13].

Results

Initially a 60 µM/mL pyruvate solution was evaluated, for LDH inhibition, in the absence of ketoprofen as a control and in the presence of 250, 500, 1000 and 1500 µg/mL of ketoprofen (Fig. 1). This provided an initial insight of the LDH inhibition activity of ketoprofen. Solutions were prepared in a phosphate buffer (pH 7.5).

A maximum of 89% LDH inhibition activity was found using 1500 µg/mL of the drug.

Different concentrations of pyruvate substrate (90, 60 and 45 µM/mL) were evaluated for enzyme inhibition in the absence of the drug i.e. control, and in the presence of 500, 750 and 1000 µg/mL of the drug, to have the parameters for the Lineweaver–Burk plot (Fig. 2) to calculate enzyme kinetics.

Ketoprofen was found to be a competitive inhibitor of LDH with an enzyme inhibition constant of Ki = 882 µg/mL (Fig. 3). The exact values of the variables shown in Figures 1, 2 and 3 are placed in Tables 1, 2 and 3, respectively.

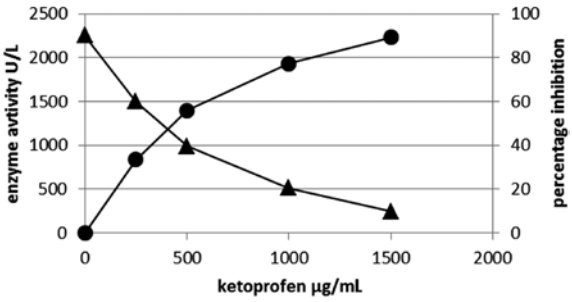

Fig. 1. Enzyme inhibition by 250, 500, 1000 and 1500 µg/mL ketoprofen against 60 µM/mL pyruvate as a substrate; percentage inhibition

Table 1. Experimental values for Fig. 1

Sr. No.	Drug Conc. (ug/mL)	Activity (U/L)	Percentage inhibition (%)
1	0	2250	0
2	250	1496	33.51
3	500	989	56
4	1000	511	77.28
5	1500	243	89.2

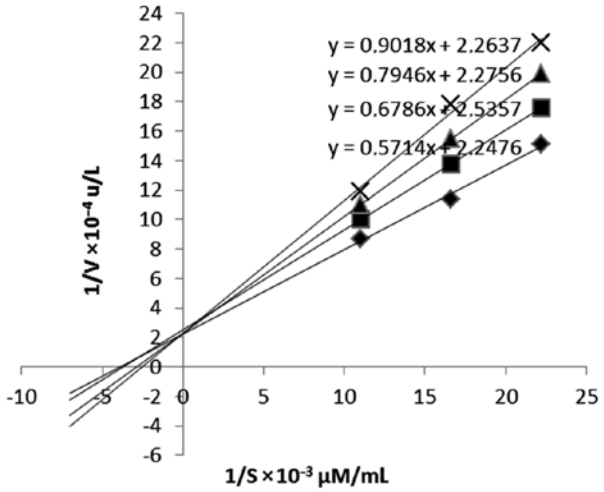

Fig. 2. The Lineweaver–Burk plot between the reciprocal of enzyme activity (1/V) and that of substrate concentration (1/S) showing competitive enzyme inhibition. – enzyme activity in the absence of ketoprofen, – enzyme inhibition in the presence of 500 µg/mL ketoprofen, – enzyme inhibition in the presence of 750 µg/mL ketoprofen and – enzyme inhibition in the presence of 1000 µg/mL ketoprofen concentration

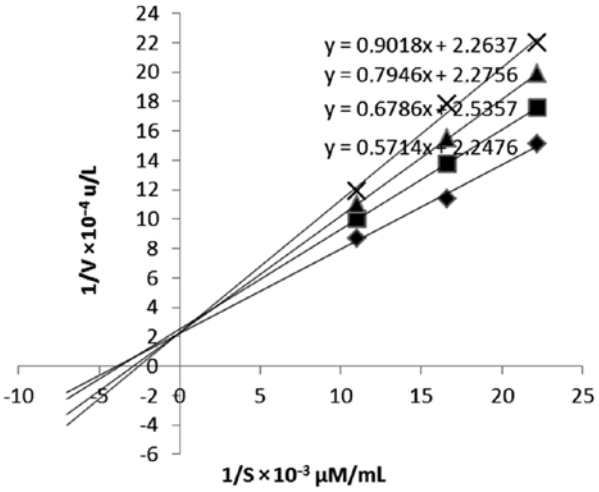

Fig. 3. Enzyme inhibition constant Ki= 882 µg/mL

Table 2. Experimental values for Fig. 2

Control	S (µM/ /mL)	1/S	V (U/L)	1/V
0	90	11×10^{-3}	1149	8.7×10^{-4}
	60	16.6×10^{-3}	877	11.4×10^{-4}
	45	22.2×10^{-3}	662	15.1×10^{-4}
Drug Conc. (ug/mL)				
500	90	11×10^{-3}	1011	9.9×10^{-4}
	60	16.6×10^{-3}	724	13.8×10^{-4}
	45	22.2×10^{-3}	588	17.6×10^{-4}
750	90	11×10^{-3}	909	11×10^{-4}
	60	16.6×10^{-3}	645	15.5×10^{-4}
	45	22.2×10^{-3}	502	19.9×10^{-4}
1000	90	11×10^{-3}	840	11.9×10^{-4}
	60	16.6×10^{-3}	561	17.8×10^{-4}
	45	22.2×10^{-3}	454	22×10^{-4}

Table 3. Values for Fig. 3

Drug Conc. (µg/mL)	Km app	Km app/Vmax
500	292	0.065633
750	349	0.078445
1000	398	0.089458

Discussion and Conclusion

LDH has an important role in the energy metabolism of platelets, so its inhibition or leakage as in the case of doxorubicin can be cytotoxic to platelets [14]. Ketoprofen has its efficacy established as an anti-inflammatory and analgesic agent [15, 16]. However its influence on platelet function i.e. thrombocytopenia, as revealed in different clinical studies, can be a concerning situation for patients using ketoprofen for long term treatment [6, 7, 17]. The dosage of ketoprofen is 200–300 mg/ /day. A single dose of 150 mg provides a plasma concentration of 15–25 µg/mL, which accumulates upon multiple dosing, resulting in enough drug concentration inside the platelets for LDH inhibition during long term use (18–20). The results of this study provide a possible explanation for thrombocytopenia associated with ketoprofen use. Thrombocytopenia is also found to be associated with Dexketoprofen, an active enantiomer of ketoprofen [21]. The platelet inhibition effects of ketoprofen can be exploited for conditions like myocardial infarction, stent thrombosis and for the drug development aspect for LDH inhibition in malaria as in the case of quinine and some azole-based compounds [22–24].

Ketoprofen is a competitive inhibitor of lactic dehydrogenase in human platelets, with an enzyme inhibition constant of 882 µg/ml. The drug inhibits 89% of LDH activity at a concentration of 1500 µg/mL. Use of ketoprofen should be avoided in patients with compromised platelet function or should be used with caution, for pain management, in patients suffering from dengue fever or thrombocytopenia as an immunogenic complication.

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