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PLA_2 INHIBITION BY LIGNOCAINE: IS IT CLINICALLY RELEVANT ?

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Key words

Decompression illness, drugs, treatment.

Abstract

The place of lignocaine administration for DCI treatment seems to be well established. The rationale for its use is a putative anti-inflammatory effect of the drug, most probably due to its ability to inhibit phospholipase A_2 (PLA_2). The purpose of the study was to quantify "in vitro" lignocaine's ability to inhibit this key enzyme and to elucidate the type of inhibition. Lignocaine inhibits PLA_2 through interaction with the enzyme-substrate complex. This occurs at plasma concentrations which are easily achievable clinically. Therefore the use of lignocaine as an anti-inflammatory drug seems warranted.

Introduction

The SPUMS Journal has published two papers on the use of lignocaine as adjuvant therapy in the treatment of decompression illness (DCI).[1,2] While both authors agree that lignocaine has a well established place in DCI therapy and that the anti-inflammatory effect of lignocaine might be the strongest rationale for using it for this purpose, there appears to be little data available on the magnitude of these anti-inflammatory effects.

Lignocaine is a known phospholipase A_2 inhibitor.[3,4] This study was to quantify "in vitro" lignocaine's ability to inhibit this key enzyme and to elucidate the type of inhibition.

Material and Methods

Blood samples were taken from nine healthy human volunteers. PLA_2 derived from the platelet membranes was incubated for 30 minutes with either TRIS buffer (native samples or controls) or lignocaine. Lignocaine concentrations of 1, 10 or 100 µg/ml (4.3; 43.0; 430 µM) were used. PLA_2 activity was measured by a modification of the method described by Flesch[5] and Sundaram,[6] while protein concentrations were determined by a modified Lowry method.[7,8] PLA_2 activities were expressed in pmol/mg protein/min. Mean values were used for statistical analysis with the Mann-Whitney rank order test. Baseline values (native activity) were considered to be 100%. All other values were expressed as a percentage of the baseline value.

For K_M and V_{MAX} determinations commercially available purified porcine PLA_2 (Sigma; Steinheim, Germany) was incubated with different substrate concentrations (0-300 µM) in the presence or absence of lignocaine (100 µg/ml = 430 µM) for 30 minutes. The PLA_2 activity was determined in a commercially available radioactive PLA_2 assay (Scintillation Proximity Assay: SPA; Amersham, Braunschweig, Germany). Data were plotted as Michaelis-Menten and Lineweaver-Burk diagrams.

Results

Lignocaine inhibits human platelet membrane PLA_2 activity in a statistically significant manner. However in the concentration range used (1-100 µg/ml) no dose dependency could be observed: the lowest concentration used led to a maximal inhibition of the enzyme (Figure 1).

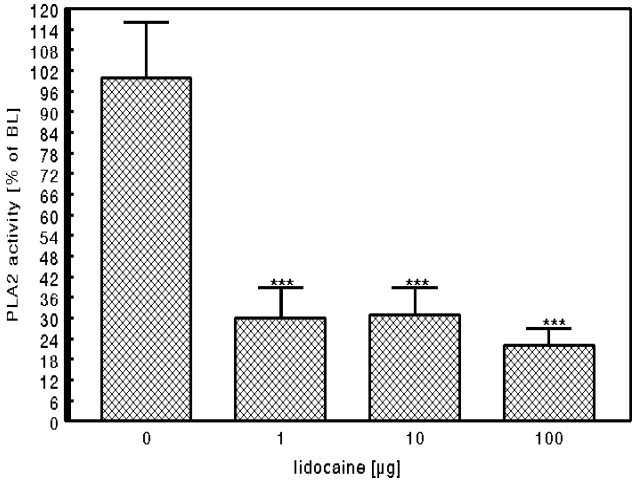

Figure 1. Lignocaine inhibits human platelet membrane PLA_2 activity in a statistically significant manner ($p \geq 0.010$). However in the concentration range used (1 - 100 µg/ml) no dose dependency could be observed.

Lineweaver-Burk representation of the data (using porcine PLA_2) suggests an interaction of lignocaine with the PLA_2 molecule and the enzyme-substrate-complex (non-competitive or mixed inhibition). The coordinates of the intersection point are x = -0.16 and y = -0.06. The inhibitor constants K_I (for the enzyme-inhibitor; EI) and K_I' (for the enzyme-substrate-inhibitor; ESI) were calculated. K_I (4,800 µM) is one order of magnitude higher than K_I' (409 µM) suggesting that the main mode of action of lignocaine is interference with the enzyme-substrate complex formation. The correlation coefficient for data determined in the absence of the inhibitor is $r_{native} = 0.96$ and for data determined in the presence of the inhibitor is $r_{Lignocaine} = 0.98$ (See Figure 2 on page 10).

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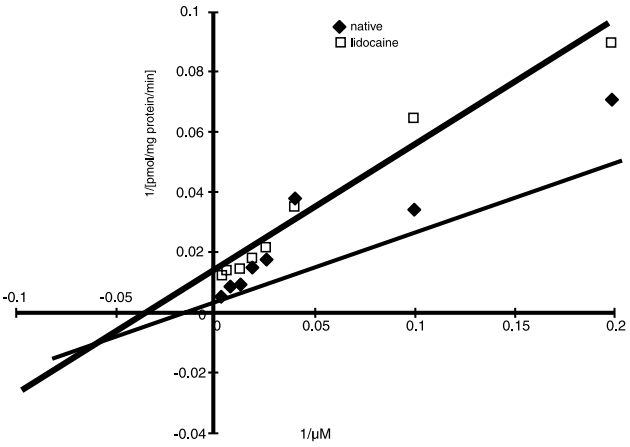

Figure 2. Lineweaver-Burk representation of the data (porcine PLA_2) suggests an interaction of lignocaine with the PLA_2 molecule and the enzyme-substrate-complex [non-competitive (mixed) inhibition].

Discussion

The effective plasma concentration range of lignocaine in humans is 1-20 µg/ml (4-80 µM). The lowest lignocaine concentration used (1 µg/ml) produced maximal inhibition of the human platelet derived PLA_2. Therefore the anti-inflammatory effect of lignocaine is easily achievable using common clinical dosages. The data derived from experiments using porcine enzyme show that the anti-inflammatory effect of lignocaine is mainly due to interaction with the enzyme-substrate-complex. The inhibitory constant K_I for porcine PLA_2 is in the 400 µM range. The most probable explanation for this value (five times higher than the upper limit of the effective plasma concentration range) is the higher sensitivity of the human enzyme to lignocaine inhibition compared with the porcine variant. Different activities/sensitivities for PLA_2 of different origins are well recognised.[9]

Conclusion

We conclude that lignocaine´s ability to inhibit PLA_2 through interaction with the enzyme-substrate-complex occurs at plasma concentrations which are easily achievable clinically. As such the use of lignocaine as an anti-inflammatory drug seems warranted.

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