Evaluation of binding capacity of aspirin to ntbi

Title:

EVALUATION OF THE BINDING CAPACITY OF ASPIRIN TO NON-TRANSFERRIN BOUND IRON – A NOVEL MECHANISM OF ACTION OF SALICYLATES.

INTRODUCTION

Aspirin, a well-known NSAID (Non steroidal anti-inflammatory drug) is commonly used for its diverse benefits- it acts as an antiplatelet agent at low concentrations, as an antipyretic and an analgesic at intermediate dose, and an anti-inflammatory agent at high dose. Traditionally, it acts by inhibiting the cyclo-oxygenase pathways which bring about its effects [1] . However, recent studies have proposed a novel mechanism of action of Aspirin and other salicylates- it can bind to iron (by acting as a chelating agent) and henceforth reduce its reactivity [2] .

Non Transferrin Bound Iron (NTBI) is free, labile form of iron found in the human plasma which is not bound to the traditional carrier, apotransferrin [3] . This form of free iron, by virtue of its charge, remains bound to other negatively bound species like albumin, citrate, acetate, and also DNA [3 , 4] . However, these species are unable to limit the redox reactions of iron and consequently ROS (Reactive oxygen species) are formed due to the Fenton reaction and the Haber Weiss Reaction [5] .

The above reactions can lead to various detrimental pathogenic changes in the body as is well known. In normal healthy individuals, the level of NTBI does not exceed 1micromol/L. The level is seen to increase in overload

conditions like haemochromatosis and thalassemia [6] . However, there have been cases where Non transferrin bound Iron has been found to be raised in the presence of unsaturated transferrin such as diabetes mellitus, liver disease and renal failure [3, 7] . Even though the role of NTBI is controversial in cardiovascular disorders, it is found to cause cardiac damage in thalassemia [8] .

We hypothesize that aspirin binding can cause the reduction of NTBI in cardiac failure patients which might contribute to its protective effects.

OBJECTIVES

1. The primary objective of this study is to demonstrate the iron binding capacity of aspirin by measuring the NTBI levels in patients suffering from cardiac failure before and after aspirin intake.
2. The secondary objective is to measure the NTBI levels in patients with cardiac failure.

METHODOLOGY

Study population: Patients who are visiting the cardiology OPD for treatment of cardiac failure

Incusion criteria : Patients who are about to start aspirin

Exclusion criteria :

1. Patients who are not willing to participate in the study
2. Patients who are already on aspirin
3. Patients who are allergic to aspirin

4. Patients on iron and vitamin supplementation
5. Patients taking iron- chelating drugs
6. Patients suffering from renal and liver disorders
7. Patients suffering from other disorders which are known to cause an increase in NTBI.

Study design

It will be a prospective observational study conducted on the patients visiting the cardiology department who have been diagnosed with cardiac failure. Blood samples of 5ml each will be collected from the patients before starting aspirin, and after 3 days, the procedure will be repeated.

Sample collection and NTBI measurement

After obtaining the informed consent, the patients will be screened for their eligibility to participate in the study. If found eligible, 5 ml of blood will be collected from each participant at baseline. The patients are usually prescribed 75 mg of aspirin. After 3 days of aspirin consumption, 5 ml of blood will be collected again one hour after the aspirin ingestion. The blood should not be anti-coagulated. The blood will be allowed to coagulate and centrifuged at 3000 rpm for 10 min. Serum will be separated and stored at minus 20 ^{0} C until further processing and evaluation.

NTBI was measured as described by Nilsson et al ^{9} . The method employs bathophenantrolinedisulphonate (BPS) which can bind with free iron (ferric ion) in a solution forming a pinkish compound. This colored compound has an absorbance at 535 nm. The absorbance will be measured in OD by the

spectrophotometric method. The glassware used will be thoroughly autoclaved and will be kept in 1 % HCl overnight to remove any metallic ion impurities. The stock solution of BPS will be made by dissolving 10 mg BPS in 1 ml of distilled water. Ferric chloride stock will be prepared by dissolving 1. 176 mg of it in 3 ml distilled water (1 mM concentration). The experiment will be performed with sterile 96 well ELISA plates and the absorbance will be measured using ELISA reader. The standard curve will be prepared by dissolving the stock ferric solution with 4 Î¼l of BPS and diluting the solution with distilled water such that a volume of 350 Î¼l will be achieved. The ferric solution will be added in increasing concentration and the corresponding absorbance will be measured such that the concentrations from 0. 25 Î¼M of ferric iron to 15 Î¼M will be covered. The readings will be taken after 30 minutes to allow for the time required to form the colored complex. Since the background variation is too much in plasma the difference in the OD before and after addition of BPS will be taken to quantify the concentration of ferric ion in the sample. To measure the ferric ion concentration in the plasma 346 Î¼l of plasma will be added in the wells and the OD will be measured. This will be followed by the addition of 4 Î¼l of BPS and after 30 min the OD will be measured again. The difference in the OD will be compared against the standard curve to obtain the quantity of the ion

The hematic parameters like total iron, total iron binding capacity, ferritin and hemoglobin will be measured both the times to look for any correlation between the NTBI and other iron parameters.

Statistical analysis

To detect a significant difference of 0. 5 Î¼M of NTBI with the SD of difference around 1 and a false positive rate of 5 %, before and after the treatment with aspirin, a sample size of 31 is required. This will be 80 % powerful in detecting the difference. The NTBI will be presented as mean (SD). The demographic parameters will be presented as mean (SD) for continuous data or as proportions for categorical data. T-test will be applied for continuous data and chi-square test for categorical data. Pearson correlation will be used for determining correlation. Multiple imputation method will be used for dealing with missing data. P < 0. 05 is considered statistically significant. SPSS v23 and R v3 will be used for statistical analysis.

Ethical justification

The study will be conducted only after obtaining a written permission from the institute’s ethics committee. Permission will be provided to start the study after through scrutiny. The patients will not be subjected to any additional intervention during the entire course of standard treatment for the disease. The study involves withdrawing a minimal amount of blood two times during the entire period of the study.

IMPLICATIONS OF THE STUDY

The study is designed to prove the binding capacity of aspirin to NTBI. It also measures the baseline NTBI levels in cardiac failure patients which can prove the elevated levels of NTBI in patients with cardiac failure. If the binding capacity of aspirin to iron is established, we can consider evaluating the

effect of aspirin in various chronic conditions where NTBI is elevated, as mentioned above.

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