DOES INDOMETHACIN, ASPIRIN OR THEIR COMBINATION HAVE DIRECT IN VITRO AND IN VIVO EFFECTS ON NORMAL ERYTHROCYTE SEDIMENTATION?

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ABSTRACT

Indomethacin (INDO) and aspirin (ASA) had been tested for their effect on erythrocyte sedimentation both in vitro and in vivo. Indomethacin (10 ug/ml) and aspirin (150 ug/ml) alone or in combination added in vitro to blood from 6 apparently normal volunteers, did not affect erythrocyte sedimentation over a period of three hours. Oral ingestion of 25-mg indomethacin capsule or two 300-mg aspirin tablets as a single oral dose or three times daily for two days, also had no effect on erythrocyte sedimentation. It is concluded that indomethacin and aspirin did not seem to have a direct effect on erythrocyte sedimentation of blood from normal subjects. Their effect on abnormally high sedimentation remains to be seen.

INTRODUCTION

Although erythrocyte sedimentation rate (ESR) is a non-specific phenomenon, its measurement is useful in conditions associated with changes in plasma proteins such as the majority of acute and chronic infections and most neoplastic and degenerative diseases[1-3]. Sudden fall may, sometime, mark the start of complications[4]. ESR may also be taken as one of several measures of the anti-inflammatory activity of drugs as non-steroidal anti-inflammatory drugs (NSAIDs) used in treatment of acute and chronic inflammatory joint disease[5-9]. NSAIDs, for example aspirin which is in addition to its well known antithrombotic effect, can interact with erythrocyte membrane and induce a local conformational changes in the lipid lalyer leading to impairment of function[10]. Changes in blood viscosity and erythrocyte function may have an important effect on erythrocyte sedime-ntation[1]. Since NSAIDs can induce such changes, it is thought rational to ask whether or not these drugs are able to alter the sedime-ntation by their direct action on the blood; an effect which may not be related to the modification of the disease process. The present study also aims, and because of the common medical practice of using two or more NSAIDs together, at investigating whether a combination of two of these drugs, in vitro and in vivo, could induce a change more than that produced by each drug alone.

MATERIALS AND METHODS

Six normal volunteers, three males and three females, age ranging from 25 to 61 years, having normal haemoglobin levels and taking no drug in the past week, gave their consent to participate in this study. Venous blood was taken in the morning and diluted with sodium citrate anticoagulant solution (trisodium citrate 109 mol/1; 32g/1) in a proportion of one volume of sodium citrate to four volumes blood. The blood was collected directly or after one-hour incubation in a water-bath at 37°C, into a clean and dry westergren tubes (Blau Brand, Germany). The tubes were held vertical in a specially made racks away from vibration and draught. Tests were normally performed at room temperature (20-25°C). The tubes were read to the nearest 1-mm the height of the clean plasma above the limit of the column of sedimenting cells in the first hour and followed for three hours, there after (cumulative sedimentation), to detect any late effect of the drugs. Drugs: Indomethacin powder (Sigma Chemicals Co.) was dissolved in absolute ethanol and diluted to

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the required concentration in phosphate buffer saline (PBS) pH 7.4. Twenty-(20) ul of the diluted solution were added to each 1 ml of blood to give a final concentration of 10ug/ml which is around the therapeutic concentration *(0.5-3 ug/ml up to 10 ug/ml)*. Indomethacin capsule, 25-mg *(Indomin, Al-Hikma, Jordan)* was given after meals as a single oral dose or given three times a day for two days provided there were no contraindication to its use. Aspirin powder *(Medex, UK)* was also dissolved in the absolute ethanol and diluted in PBS, pH 7.4. Twenty-(20) ul of the final dilution was added to each 1 ml of blood to give the required concentration of 150 ug/ml *(aspirin therapeutic concentration ranges from 20-100 ug/ml for analgesic and 150-300 ug/ml for anti-inflammatory effects)*. Two aspirin tablets *(Aspirin Adult tablet, 300 mg, SDI, Iraq)* were given after meal either as a single oral dose or three times daily for two days. For controls of in vitro studies, 20 ul of PBS in which ethanol is added in amount equal to that used in the drug solutions. This was increased to 40 ul to adjust for the use of the two drugs together. Student t-test was used for statistical analysis.

RESULTS

1. In vitro effect of indomethacin (10 ug/ml), aspirin (150 ug/ml) or their combination on normal erythrocyte sedimentation. There was no statistically significant effect of indomethacin or aspirin, or their combination, added in vitro on the sedimentation of normal erythrocytes when followed for three hours after incubation with these drugs (table 1). The erythrocyte sedimentation rate (ESR) ranged from 6 mm/h to 25 mm/h. The volunteer with ESR of 25 mm/h is a sixty-one year woman; her haemoglobin was 12.5 g/dl with normochromic, normocytic blood film.

2. The effect of a single oral dose of indomethacin, aspirin or their combination on erythrocyte sedimentation of blood from normal volunteers. Aspirin did not seem to have an effect on erythrocyte sedimentation measured 2 hours after ingestion of two 300-mg tablets by four volunteers (table 2). Similar conclusion was drawn after ingestion of one 25 mg capsule of indomethacin by four volunteers *(5.3, 12.3, 25.3 mm before, and 5, 15.7, 25.7 mm 2 hours after ingestion measured at the 1st, 2nd, and 3rd hour of erythrocyte sedimentation respectively)*.

3. The effect of frequent administration of indomethacin and aspirin on erythrocyte sedimentation of normal volunteers. Three subjects volunteered to take indomethacin; one 25mg capsule three times daily for two days. Another three subjects took aspirin; two 300mg tablets three times daily for two days. A washout interval of one week was required if the same subject volunteered to take the two drugs in sequence. No clear effect of a 2-day ingestion of these drugs on erythrocyte sedimentation when it is compared to its values before drug ingestion (table 3).

DISCUSSION

The two non-steroidal antiinflammatory drugs *(NSAIDs)* used in this study may have slightly different action on the cyclooxygenase enzyme. Aspirin acetylates this enzyme and inhibits it irreversibly[11,12]. Although, erythrocytes are thought to be free from the enzyme system responsible for prostaglandin synthesis, aspirin has been shown to induce local conformational changes in the membranes of erythrocytes and platelets which may lead to impairment of their function[10]. However, indomethacin and aspirin used singly or in combination did not produce a clear effect on the sedimentation of erythrocytes when they are added in vitro or ingested orally. This could indicate that changes in the ESR after the use of these drugs may be due to changes in disease activity rather than a direct effect of the drugs on erythrocyte sedimentation. Although each of our volunteers acts as his own control, two of them had relatively rapid sedimentation compared to others. Still, no effect of aspirin and indomethacin on their erythrocyte sedimentation. Several factors can affect the extent of erythrocyte sedimentation. One of them is the important phenomenon of rouleaux formation which is controlled by the concentration of fibrinogen and other acute-phase proteins and also by immunoglobins[1]. It is retarded by albumin and is extremely slow in defibrinated blood. The ratio of red cells to plasma i.e. PCV and plasma viscosity are other factors. Anaemia encourages rouleaux formation and accelerates sediment-ation. Similarly ESR is higher in women than in men,

and there is progressive increase with age; ranges for men extend from 10 to 30 mm in those over 70 years and for women from 12 to 35-mm[1]. Finally, sedimentation is normally accelerated as the temperature rises. The results of the present study support Gotzsche[13] meta-analysis findings of 130 clinical trials, that several variables may be ommitted from clinical trials; ESR is one of them. The 95% confidence interval for the difference between NSAIDs and placebo is –1.5 to 3.4 mm/hour, which is not statistically significant. Combination of two NSAIDs can lead to increased toxicity with no additive beneficial effect[14]. Combination of indomethacin and aspirin added in vitro or administered, as a single oral dose did not produce any significant change in erythrocyte sedimentation. Although, the present study showed that indomethacin and aspirin to have no effect on normal sedimentation, it remains to be seen if these NSAIDs can affect the sedimentation when it is accelerated, or their effect will be evident only after being administered for longer periods.

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