DOES THE ASSOCIATION DIPYRIDAMOLE-ASPIRIN ONLY ACT BY A FUNCTIONAL SYNERGISM?

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Abstract—1. Some of the antiplatelet effects of the dipyridamole–aspirin association may be explained by a functional (additive) synergism, but other effects by a potentiation synergism.
2. Platelet adhesivity and aggregation induced by ADP, adrenaline and collagen, and malondialdehyde production, are tests in which dipyridamole and aspirin shows a potentiation synergism.

INTRODUCTION

One of the basic principles of pharmacology refers to the fact that two drugs should be associated only when a reduction of the possible side effects of each one is likely to be achieved, or else when a synergism is developed, i.e. the effect that is obtained with the single drugs (Goodman et al., 1985). When two drugs are associated, a potentiation synergism is expected rather than a simple addition of effects (functional synergism).

On this basis, we have supposed that the association dipyridamole (DIP) plus a moderate low dose of aspirin (ASA) was likely to best inhibit platelet function. We have based the study on the results that have already been obtained and published by our group, in both healthy volunteers (Aguilar et al., 1985; De la Cruz et al., 1989) and diabetic patients (Aguilar et al., 1986; De la Cruz et al., 1986), who were treated with DIP, ASA and their association. The method employed for the study of the pharmacologic synergism was the one described by Poch and Holzman (1980) and Poch (1981, 1982, 1984).

MATERIALS AND METHODS

Design of the study

We have based our study on the data previously published by our group, referred to healthy volunteers and insulin-dependent diabetic patients without any clinically observable microangiopathy.

In all the cases each treatment lasted 15 days. Between periods, 15 days of wash-out were observed. The doses were as follows: 75 x 3 mg/day of DIP, 50 x 3 mg/day of ASA, and the association at the same doses. We introduced all the individual data of each platelet test for each patient and for each period of treatment (all people were submitted to the three periods of treatments) on a Lotus-Symphony program (Lotus Development Co., Version 1.1; 1984-85).

Platelet tests

The following tests were carried out at the beginning and the end of each period of treatment: (a) platelet adhesivity to glass (Hellem, 1970); (b) platelet aggregation in platelet-rich plasma (Born, 1962), using ADP, adrenaline, collagen and arachidonic acid as inducers; and (c) platelet lipid peroxidation (malondialdehyde production) (MDA) (Smith et al., 1976).

Numerical analysis

We have used the method described by Poch and Holzman (1980) and Poeh (1981, 1982, 1984), according to which the effect of the association of two drugs, E(a + b), is higher than those obtained separately \(E(a) + E(b)\), there is a synergic effect that may be either equal to the expected one (functional synergism) or higher (potentiation synergism). The advantage of this method is that may be applied to \textit{ex vivo} studies, in which it is not necessary to use equipotent doses of drugs (Poch and Londong, 1985).

In order to evaluate the above alternative, we calculated the following parameters on the basis of the data of our casuistics:

(a) observed effect (E) of each treatment period, defined by:

\[ E = 1 - \frac{\text{posttreatment value}}{\text{pretreatment value}}; \]

(b) expected or calculated effect \(E'\), defined by:

\[ E'(\text{DIP} + \text{ASA}) = E(\text{DIP}) + E(\text{ASA}) \]
\[ - E(\text{DIP}) \times E(\text{ASA}). \]

The maximum possible value of \(E\) and \(E'\) is 1.

The above authors consider that when \(E(\text{DIP} + \text{ASA}) = E'(\text{DIP} + \text{ASA})\), there is a functional synergism, but when \(E(\text{DIP} + \text{ASA}) > E'(\text{DIP} + \text{ASA})\), there is a potentiation synergism. Finally, when \(E(\text{DIP} + \text{ASA}) < E'(\text{DIP} + \text{ASA})\) there is no synergism.

The Wilcoxon test for matched ranked pairs was used for making the comparisons \((E vs E')\), with two-tail reading and by means of the Epistat statistical program (T. L. Guftason, U.S.A., 1985). Differences with \(P < 0.05\) were considered significant.

RESULTS

Figure 1 shows differences between parameter \(E\) and \(E'\) on the tests carried out in healthy volunteers. Figure 2 shows the same evaluation in diabetic patients. Also we determined plasma \(\beta\)-thromboglobulin levels, but neither DIP, ASA nor DIP + ASA modified it. Serum thromboxane B-2 was inhibited 99.2% by ASA alone, and \(E(\text{DIP} + \text{ASA}) = E'(\text{DIP} + \text{ASA})\).
In healthy volunteers $E(DIP + ASA)$ was higher statistically than $E'(DIP + ASA)$ on: adhesivity to glass and aggregations induced by ADP and collagen; in diabetic patients: aggregations induced by ADP, adrenaline and collagen and platelet malondialdehyde production.

**DISCUSSION**

Poch and Holzman (1980) and Poch (1981, 1982, 1984) have proposed a method for the analysis of the synergism between two drugs *ex viva* at such doses that when they are administered separately determine a maximum effect. This method allows for the study of the synergism without any recurrence to alterations of the therapeutic doses; furthermore, it allows for the evaluation of the influence of pharmacokinetic and pharmacodynamic factors in their "natural" site of action.

Concerning the association dipyridamole-aspirin, the test is quite adequate, since aspirin is metabolized in the liver in salicylic acid which interacts at the platelet and vascular cyclooxygenase with ASA (Pederson and Fitzgerald, 1984; Cerletti *et al.*, 1984; Jakubowski *et al.*, 1985; Brandon *et al.*, 1986; Alcorn *et al.*, 1987, among others). The importance of the said interaction is such that the above authors suggest that the aspirin dilemma is more pharmacokinetic than pharmacodynamic (Cerletti *et al.*, 1984). This factor should be taken into account when performing the Poch test, but is not the case for the *in vitro* tests.

Results obtained in healthy volunteers (Fig. 1) prove that there is a potentiation synergism between DIP and ASA in platelet adhesivity to glass, which is in agreement with Rajah *et al.* (1979). Seemingly, there is an interaction between both drugs in the early stages of the platelet activity. When they were used separately, inhibitory effects of 29% for DIP and 23.4% for ASA were obtained (Aguilar *et al.*, 1985). These data are in agreement with the potentiation synergism found on platelet aggregation induced by ADP, because platelet fibrinogen receptors are in relation with the early steps of platelet activation, and furthermore it exhibits a close functional relationship with the platelet ADP receptor (Marguerie *et al.*, 1980).

DIP increase AMPc levels by inhibiting AMPc-phosphodiesterase (Rozemberg *et al.*, 1971). For that
Dipyridamole-aspirin synergism

Fig. 2. Effect on different platelet tests in diabetic patients of the dipyridamole-aspirin association calculated for functional synergism expected (C) and observed (O). (P-Values of Wilcoxon matched pairs signed rank test.)

reason the inhibition of the adenylcyclase derived from ADP (Marguere et al., 1980) would not provoke a considerable aggregatory effect; this effect might diminish the binding of fibrinogen to its receptor.

On the other hand, aspirin inhibits the negative effect of arachidonic acid-derived products on AMPc levels, because it blocks the cycloxygenase (Vane, 1971). Those effects can explain a possible potentiation synergism. The explanation for collagen-induced aggregation is similar, but the inhibition of the cycloxygenase pathway might play a more major role than the AMPc stimulus derived from the action of DIP.

The results obtained in diabetic patients (Fig. 2) confirm those obtained in healthy volunteers. However, differences are less important and it is due perhaps to the higher pretreatment values in diabetics than in healthy people (Aguilar et al., 1986; De la Cruz et al., 1986).

Moncada and Korkut (1978) supposed that in vitro, DIP increases prostacyclin production, which produces an increase in platelet AMPc levels. On the other hand, moderate low doses of aspirin block platelet thromboxane synthesis and maintain unaltered the vascular prostacyclin production (De la Cruz et al., 1986). For that reason, the association DIP-ASA would exert a potentiation effect at two different but related platelet levels, as we could observe when applying the Poch test to our results.

REFERENCES


