HUMAN PLATELET FUNCTION AS A MODEL FOR INVESTIGATING THE CLINICAL EFFICACY OF CHLORPROMAZINE

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- 1 Enhancement of platelet aggregation response (PAR) to 5-hydroxytryptamine (5-HT) in some schizophrenic patients receiving chlorpromazine (CPZ) may provide a biological index for the efficacy of this drug.
- 2 In a double-blind study 33 schizophrenic patients hospitalized following their first psychotic breakdown were followed up clinically with concurrent assessment of their PAR to 5-HT. The patients followed a standardized treatment schedule with (CPZ) as the sole antipsychotic medication.
- 3 Twelve patients (Group A) developed an enhanced biphasic 5-HT PAR, within 2–3 weeks and improved clinically by the sixth week. In most cases, the appearance of the enhanced biphasic PAR preceded clinical improvement. Twenty-one patients (Group B) did not have enhanced biphasic PAR to 5-HT by the sixteenth week of treatment. However, twelve subjects from this group showed clinical response to CPZ by the end of this period, while the remaining patients did not improve.
- 4 The enhanced PAR to 5-HT in Group A discriminated best between good and bad outcome cases when Feighner's research diagnostic criteria were used. We could not confirm the previous reports of platelet aggregation response to dopamine in pre- or post-chlorpromazine treatment.

Introduction

Chlorpromazine (CPZ) and several of its metabolites have been shown to inhibit, *in vitro*, platelet aggregation induced by the addition of 5-hydroxytryptamine (5-HT) (Boullin, Grahame Smith *et al.*, 1975). On the other hand, enhancement of platelet aggregation response (PAR) to 5-HT has been found to occur in some schizophrenic patients treated chronically with CPZ and fluphenazine (Boullin, Wood *et al.*, 1975). Consequently it was suggested that this could be of value as a biological index to monitor the clinical efficacy of phenothiazine neuroleptic drugs. The rationale lies in the similarity between receptors on blood platelets and CNS monoaminergic neurons (Pletscher, 1977) on the one hand, and the failure in using plasma levels of CPZ or metabolites as an index of efficacy on the other.

However, the initial enthusiasm of the Oxford group of investigators has been followed by scepticism following their lack of success in replicating their initial findings (Boullin *et al.*, 1979). For the last two years, we have been engaged in repeating the studies of Boullin, Wood *et al.* (1975) though with a somewhat different clinical design. Thus, we have used several diagnostic systems in the screening of our

cases and, unlike Boullin, Wood *et al.* (1975), we have concentrated our efforts on 'first-breakdown' schizophrenics. We have decided to exclude the chronic cases, as we believe that a long history of previous treatment regimes and factors associated with chronic disease might confound our results. (Boullin *et al.*, 1978; Hefez *et al.*, 1979).

Methods

Clinical

Every adult (19–40 years) admitted to the psychiatry department in Rambam Hospital and suspected of having a schizophrenic episode for the first time in his life was included in this research. Before hospitalization, the patient must have taken no antipsychotic medication and during the first days following admission (the screening phase) only diazepam was prescribed.

The initial screening consisted of a full clinical assessment and a determination of PAR. At the initial phase and throughout the whole project,

clinical and pharmacological assessments were carried out by separate teams who had no knowledge of each other's findings. The clinical data were in a check-list form which included Bleuler's Fundamental Symptoms, Schneider's FRS and Feighner's research diagnostic criteria for schizophrenia. In addition, the degree of pathology was assessed using the Hopkins Psychiatric Rating Scale (HPRS) (Derogatis, 1976). Four subscales were chosen as our clinical parameters for degree of pathology. These are: Global Pathology Index (GPI), Psychoticism, Conceptual Dysfunction, and Paranoid Ideation. The GPI is a 9 point scale while the three other subscales are 7 point scales. These subscales have anchoring definitions and satisfactory reliability. The patients included in this research followed a standardized treatment plan with CPZ as the only antipsychotic medication administered on a flexible dose design. Additional treatment included E.C.T. given on the average once a week for 10 treatments, trihexyphenidyl HCl up to 10 mg daily to counteract extrapyramidal side-effects, and nitrazepan (5 mg) as an hypnotic. Any possible influence of E.C.T. on PAR to 5-HT was excluded before carrying out our experimental design.

Platelet aggregation response (PAR)

Clinical status and PAR were assessed every week. The method for measuring PAR to 5-HT, adrenaline, dopamine and ADP in citrated blood has been described by Baumgartner & Born (1968). Blood (20 ml) is collected in plastic tubes containing 3.5% (w/v)

sodium citrate and platelet rich plasma (PRP) is prepared by centrifugation at 200 g for 10 min. The supernatant (PRP) is carefully removed and placed in plastic tubes which are kept at 37°C until platelet aggregation is performed. The turbidity of the PRP is very high. As a blank of zero turbidity value a platelet poor plasma (PPP) is prepared by centrifugation of PRP at 2000 g for 10 min. The supernatant, PPP, is used as the blank. Standard solutions of monoamines and ADP are used at concentration of 1–20 μM each.

The aggregation responses were recorded with the platelet aggregometer coupled to an x-y pen recorder. The maximal aggregation response to 5-HT is expressed here in terms of ratio of areas under the curve showing the PAR to 5-HT to the PAR response to ADP expressed in percentage. An enhanced response to 5-HT will be indicated by a higher value of this ratio. The enhancement of 5-HT aggregation results in an irreversible or biphasic response, similar to that of ADP, in most cases. All values are expressed as mean $\pm$ s.d.

Results

Chlorpromazine therapy and platelet aggregation

Preliminary double-blind studies on eleven acute schizophrenic patients showed that those patients who developed a biphasic PAR to 5-HT (Figure 1) during CPZ treatment had a more favourable outcome than patients whose PAR to 5-HT remained unchanged (Hefez, Oppenheim & Yodim, 1980).

Figure 1 The platelet aggregation responses (PAR) to 5-hydroxytryptamine (10 μM) in one schizophrenic patient (B.H., female, age 21 years) who showed clinical response to chlorpromazine (CPZ) within 2 weeks of commencement of treatment. (a) Aggregation response before treatment; (b) aggregation response 3 weeks after start of CPZ administration (400 mg day⁻¹).

Figure 2 The changes in 5-HT-induced PAR in 33 acute schizophrenics receiving only chlorpromazine. (●) enhanced aggregation showing biphasic 5-HT PAR ($n = 12$) and (○), no enhanced aggregation ($n = 21$). The ratio of 5-HT:ADP PAR before the commencement of treatment with CPZ were similar in both groups. The s.e. mean is not shown for the sake of clarity. The enhancement of aggregation observed within 2 weeks of treatment is similar to that which has been reported by Boullin *et al.* (1978).

The present data refer to 33 patients of whom 32 rigorously adhere to all our criteria. We have decided to include one more patient, though the HPRS has been incomplete, because the biphasic PAR to 5-HT coincided clearly with a marked clinical improvement in the third week of treatment. Two additional schizophrenic cases had a biphasic PAR to 5-HT before commencement of treatment. In both, the clinical improvement was rapid (although these cases are not included here, it would be interesting to investigate the relationship between a baseline irreversible PAR to 5-HT and the clinical response to CPZ). The 33 patients (24 male and 9 female) forming the substance of this report satisfied one or more of the three diagnostic criteria for schizophrenia and had had no previous psychotic breakdown (except for one patient who suffered from a short breakdown more than 10 years previous to this study). None of them had received antipsychotic medication before the present hospitalization and their average age was 24.4 ± 6.0 years. All patients had a baseline reversible PAR to 5-HT with the mean of 5-HT/ADP aggregation ratio of 13.7 ± 8 (s.d.). This value is not significantly different from the ratio of PAR to 5-HT/ADP in normal untreated subjects (15.4 ± 7 (s.d.), $n = 45$). Eleven patients (Group A) developed enhancement of PAR to 5-HT during CPZ treatment which culminated in the appearance of a biphasic

(irreversible) response similar to the PAR normally observed for ADP (Figure 1). The ratio of 5-HT/ADP PAR in Group A patients after 2 weeks of CPZ treatment was 49 ± 11 (s.d.), which was significantly different ($P < 0.01$) from their pretreatment baseline ratio (Figure 2). No similar enhancement of PAR to 5-HT was recorded in the remaining 21 patients (Group B) during the period of treatment with CPZ (Figure 2). The aggregability of platelets by E and ADP was not affected, so that the chlorpromazine effect to this extent is specific for 5-HT. Unlike Boullin *et al.* (1978), we could not demonstrate platelet aggregation response to dopamine either in platelets from normal subjects of CPZ treated patients.

Comparison of the Global Pathology Index of the two sub-groups shows that patients developing enhanced PAR to 5-HT (Group A) fared better during the first 6 weeks of treatment (Figure 3). However, the difference is only significant on Global Pathology. On the psychoticism scale it was short of the 5% significance level. The difference was even less marked on the conceptual dysfunction and paranoid ideation parameters. In order to carry our analysis further, we attempted to classify our patients into 'improved' and 'not improved'. The criterion for clinical improvement was arbitrarily chosen as 'a decrease of at least 2 points in the global pathology

Figure 3 The clinical improvement of acute schizophrenic patients with (▨) and without (□) a biphasic 5-HT PAR during CPZ treatment. The numbers above the columns indicate the number of patients in each group. In the sixth week from the start of treatment, CPZ had to be stopped in five patients, either because of intolerance (one patient) or because of deterioration in mental condition (four patients). The results are expressed at ± 1 s.d. and global pathology in patients showing enhanced biphasic 5-HT PAR (Group A) was significantly lower at week 3 ($P < 0.05$) and week 6 ($P < 0.01$) of CPZ treatment. Although the global pathology of Group B (PAR not biphasic) was lower at the sixth week, the values were not significantly different from the pretreatment period.

score provided that 'at the end of 6 weeks the final score is not higher than 3'. A score of 3 on the Global Pathology Index indicates a mild degree of dysfunction, mainly subjective. Accordingly, 24 patients of our sample showed improvement sometime during the 6 weeks, while in 9 patients the psychiatric status remained unchanged or worsened. All group A patients having a biphasic PAR to 5-HT were in the 'improved' group. Thus, while clinical improvement was present in every patient with a biphasic PAR to 5-HT, not every case that clinically improved developed a biphasic PAR. Furthermore, the appearance of an irreversible platelet aggregation response to 5-HT preceded the clinical improvement in 9 out of the 11 cases (Group A) (as mentioned, one case had an incomplete HPRS and was therefore excluded from this analysis). This is statistically significant at the 5% level (binominal test $P = 0.33$ one tailed test). Our next step was to investigate a possible association between certain diagnostic criteria and the PAR on one hand and clinical improvement on the other. The Venn diagrams (Figure 4) show the overlap between the three diagnostic systems as they were found in our patients. As is seen, the Bleulerian criteria were present in all our cases. This is quite understandable

taking into account the ambiguity and flexibility of the Bleuler's fundamental symptoms. The proportion of Bleulerian schizophrenics who concomitantly show Schneider's FRS corresponds to the findings of other authors (Carpenter, Strauss & Bartko, 1974; Freizer & Jeffrey, 1979). Similarly, the overlap between the Feighner and Schneider's groups are consistent with Koehler's findings (Koehler, 1978). By comparing the outcome of these three sub-groups as related to their platelet aggregation response, it is obvious that a biphasic PAR to 5-HT predicts improvement within 6 weeks from the commencement of CPZ treatment in all first breakdowns, whichever of the three diagnostic systems is used.

On the other hand, the absence of a biphasic response might be of some use in predicting lack of efficacy of CPZ only in Feighner schizophrenics ($P < 0.05$). Thus the PAR to 5-HT is of greater discriminative value when Feighner's research diagnostic criteria are used. There was no significant difference between the mean age of patients who developed a biphasic PAR to 5-HT (mean 23.6, s.d. 5.6) and that of patients with no biphasic response (mean 24.8, s.d. 6.5). However, patients who did not develop a biphasic response and showed clinical improvement

Figure 4 The clinical outcome of 33 acute schizophrenic patients diagnosed according to Bleulerian (B), Schneiderian (S) and Feighner's (F) criteria. All patients have been on CPZ treatment only. Twelve patients developed a biphasic PAR to 5-HT and 21 subjects showed no enhancement (second phase) of PAR to 5-HT.

were older than those with a biphasic PAR, although the difference was short of the 5% level of significance. On the other hand, non-improved patients were significantly younger than the improved, non-biphasic sub-group. It seems that a biphasic aggregation response, somehow counterbalances the deleterious effect of young age on the course of schizophrenia.

Similarly, although poor premorbid adjustment was present equally in both sub-groups, it tended to be associated more with bad clinical response in the non-biphasic sub-groups. As to sex, we found no relation between sex and clinical response to CPZ, although the sex ratio was significantly different. Thus, in the group with biphasic PAR there was an equal number of males and females, while in the non-biphasic group the ratio was 6:1 with a preponderance of females. Considering the fact that CPZ dosage might be related to the difference in PAR and/or clinical outcome, we compared the mean daily dose curves in both groups (Figure 5). During the first 4 weeks of treatment the two curves are parallel and close to each other; however, in the last 2 weeks the two curves diverge, with the non-biphasic PAR group

receiving a significantly higher daily dose. This is probably a consequence of the lack of improvement necessitating administration of a higher dose for a longer period.

The effect of non-phenothiazine neuroleptics on platelet aggregation

In addition to the 33 patients on CPZ we have followed the PAR in 8 patients who received other non-phenothiazine drugs (e.g. haloperidol) or were non-schizophrenic but treated with CPZ for other disorders. Of these one patient suffered from a degenerative brain disease with a schizophrenic-like picture. In all these patients, the PAR to 5-HT was unchanged throughout the 16 week follow-up period. Further studies with non-phenothiazine drugs are required to establish whether the biphasic 5-HT PAR obtained with CPZ (in this study) and with fluphenazine (Boullin *et al.*, 1978) is the property of the phenothiazine class of neuroleptics, as stated by Boullin *et al.* (1978).

Figure 5 The average daily dosage of CPZ in schizophrenic patients showing enhanced 5-HT-induced PAR (●, $n = 12$) and patients not showing such a response (Group B, ○, $n = 21$). There is no significant difference between the two groups.

Discussion

The enhancement of the PAR to 5-HT in certain schizophrenic patients receiving CPZ is probably due to certain changes in the platelet structure of these patients. It has been shown that plasma from such patients has no enhancing effect on platelets from untreated normal subjects. The time lag between the commencement of CPZ treatment and the appearance of a biphasic response (on average 14 days) may be due to the fact that CPZ and/or its metabolites affect the newly formed platelets and a sufficient number of such platelets have to accumulate before the enhancement of aggregation can be detected. The finding that only some schizophrenic patients develop such a response when treated with CPZ favours the assumption that there exists a biologically distinct group of schizophrenic patients and such a group cuts across the various diagnostic systems used, viz. Bleuler's, Schneider's and Feighner's. Consequently, every one of the three diagnostic groups is probably biologically heterogeneous.

Moreover, at the present stage of our research we believe that the appearance of a biphasic PAR to 5-HT within the first 2 weeks from the commencement of CPZ treatment is always followed by substantial clinical improvement. This may not be the case with chronic CPZ treatment where the appearance of biphasic PAR to 5-HT may have no relation to clinical outcome (Boullin *et al.*, 1978).

Although we found no difference in CPZ doses given to patients who developed a biphasic PAR to 5-HT and those who did not develop this response, this does not preclude that high CPZ doses given for longer periods may eventually lead to such biphasic PAR to 5-HT. In such a case, we can assume that there is a greater susceptibility of the platelets in those schizophrenics who show better clinical response to CPZ.

More recently Baldacci *et al.* (1980) have observed that chronic daily treatment of rabbits with CPZ (2 mg/kg) up to 4 weeks results in increased aggregability by 5-HT and not by ADP. Thus to this extent the CPZ effect, as in our studies, was specific for 5-HT.

The mechanism whereby platelets from schizophrenic patients treated with CPZ show enhanced platelet aggregation to 5-HT is not understood. Boullin *et al.* (1978) have suggested that CPZ therapy may affect megakaryocytosis to produce an abnormal population of platelets which show enhanced responses to 5-HT.

The effect on 5-HT induced aggregation by CPZ could simply be explained by an increase in the number of platelet receptors that are specific for 5-HT. This could occur in the megakaryocytes from which platelets are derived. And by analogy with other neuroleptic induced receptor supersensitivity, such an increased receptor synthesis could be a consequence of the blocking *in vivo* of 5-HT receptors by

CPZ (Heal *et al.*, 1976) for which it has a high affinity (Graf & Pletscher, 1979). This would show itself in the case of platelets as an increase in aggregation by 5-HT. It is more than interesting to note that haloperidol, which has high and low affinities for dopamine and 5-HT receptors respectively, did not induce biphasic 5-HT PAR in patients receiving this drug and is also a weak inhibitor of 5-HT-induced PAR as compared to CPZ (Goldstein & Youdim, submitted for publication).

Animal (Baldacci *et al.*, 1980) and human *in vitro* studies with platelets have shown that 5-HT enhanced aggregation is not a direct effect of CPZ or its metabolites (Boullin *et al.*, 1978; Oppenheim *et al.*, 1980) but is associated with a change in the platelet itself. However, one must also consider the possibility that CPZ, like other neuroleptics, increases the turnover of catecholamines in the circulation (Seeman, 1977). Their presence in platelet rich plasma can be a source of enhancement of 5-HT aggregation. Mills & Roberts (1967) and more recently Boullin *et al.* (1978) have shown synergism between non-aggregating concentration of noradrenaline and 5-HT leading to enhancement of 5-HT aggregation response very

similar to aggregation response observed for ADP. Whatever the mechanism of enhanced platelet aggregation of 5-HT in CPZ treated patients, further work is necessary not only to elucidate the action of CPZ but also to further investigate the use of platelet aggregation as a predictive test of sub-groups of schizophrenic patients treated with neuroleptics.

In summary, when a biphasic PAR to 5-HT appeared in the first 3 weeks of CPZ treatment it was associated in all cases with clinical improvement. In nine out of ten cases this irreversible PAR preceded the detection of Clinical Improvement. The biphasic PAR discriminates the good from bad outcome cases, mostly when Feighner Research criteria are used, less so when Bleuler's Fundamental symptoms are used and least in the case of Schneider's FRS. A side issue to this is the possibility that Feighner's schizophrenia might consist of two biologically distinct subgroups.

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