Immunological assessment of patients with rheumatoid arthritis — evaluation of the effects of propranolol

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Summary

Some humoral and cellular immune functions were evaluated in a group of 10 patients with rheumatoid arthritis before and after 3 months of treatment with the β-blocking agent propranolol. Humoral parameters measured were serum immunoglobulins including IgE, auto-antibodies, C-reactive protein, total haemolytic complement activity and the complement components C3 and C4. Cellular functions assessed were polymorphonuclear leucocyte chemotaxis, phagocytosis and post-phagocytic nitroblue tetrazolium reduction, hexose monophosphate shunt activity and myeloperoxidase-mediated iodination of ingested protein; lymphocyte transformation to the mitogens phytohaemagglutinin and concanavalin A was also investigated. No alteration of humoral factors and neutrophil functions was observed following propranolol administration (40 mg 3 times a day), but lymphocyte transformation was significantly increased. Improved lymphocyte function did not correlate with clinical improvement.

Propranolol possesses a number of activities, probably unrelated to β-receptor blockade, which suggested the potential usefulness of this agent in the treatment of rheumatoid arthritis (RA). These are: (a) immunostimulatory activity in vivo and in vitro; (b) in vivo inhibition of release of rheumatoid factor (RF) by cultured human blood lymphocytes; and (c) inhibition of the myeloperoxidase (MPO)/H₂O₂/halide system in vitro, which is also a property of certain non-steroidal anti-inflammatory agents used in the treatment of RA. Furthermore, we had heard anecdotal accounts of patients whose RA or other connective tissue diseases had improved following propranolol therapy for co-existent hypertension or angina pectoris. For these reasons a pilot study was undertaken to assess the immunological status of 10 patients with RA before and during propranolol administration. No attempt was made to carry out a formal clinical trial, but changes in the subjects' clinical condition were compared with the laboratory findings.

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Patients and methods

Informed consent was obtained from 10 White patients, 4 males and 6 females, whose ages ranged from 20 to 71 years. All fulfilled the American Rheumatism Association criteria for the definition of classic RA and had been under treatment for more than 6 months, exhibiting active disease in a steady state while on a conventional regimen of non-steroidal anti-inflammatory agents. All had failed to respond to either gold or d-penicillamine treatment. None was on steroids.

The β-receptor blocking agent was given in a fixed dose of 40 mg 3 times a day in an open trial over a 12-week period. Clinical evaluation was made at the outset and after 1 and 2 months of propranolol therapy. Immunological testing was performed before and after 3 months of therapy.

The following parameters were used to determine the clinical condition of the patients: (i) number of swollen tender joints; (ii) duration of morning stiffness; (iii) grip strength (mmHg); (iv) joint movement, graded as: 0 — normal, 1 — slight impairment, 2 — moderate impairment, and 3 — severe impairment; (v) degree of disability, graded as above; (vi) patient evaluation, graded as: 0 — considerable improvement, 1 — slight improvement, 2 — no improvement, and 3 — worsening of the condition; (vii) doctor’s evaluation, graded as above; and (viii) side-effects (none were in fact complained of).

Cellular studies

Neutrophil motility. Neutrophils were obtained from heparinized venous blood (heparin 5 U/ml). After sedimentation at 37°C the buffy layer was centrifuged at 250 g for 10 minutes. The resultant cell pellet was washed twice with Hank’s balanced salt solution (HBSS) (Grand Island Biological Co., NY, USA), supplemented with Hepes (Sigma, St Louis, Mo., USA) 1 g/l to give a final pH of 7.2. Polymorphonuclear lymphocytes (PMNLs) were resuspended to a final concentration of 5 x 10⁶/ml. Two leuco-attractants were used in the study: (i) fresh pooled normal sera activated with bacterial endotoxin (Escherichia coli 0127:B8, Difco, Detroit, Mich., USA) 100 µg/ml; and (ii) endotoxin-activated autologous serum (EAS). Both types of EAS were incubated for 30 minutes at 37°C followed by an eightfold dilution with HBSS. Neutrophil motility was assessed using a modified Boyden chamber as described by Wilkinson, which utilizes 0.2 ml of patient or control neutrophils in the upper chamber separated from 0.8 ml of the leuco-attractant by a membrane filter with a pore size of 5 µm (Millipore Corp., Bedford, Mass., USA). Chambers were incubated at 37°C for 3 hours, after which the filters were fixed in methanol, detached and stained in haematoxylin. The average number of cells reaching the lower surface of the filter was determined and expressed as an average for triplicate filters.

Phagocytosis. This was assessed as previously described by the ingestion of Candida albicans. Briefly, to 0.5 ml of PMNL suspension (6 x 10⁶ PMNLs/ml) were added 0.1 ml fresh serum, 0.1 ml C. albicans suspension (9 x 10⁷/ml) and 0.3 ml HBSS. The mixture was incubated on a turntable at 37°C for 30 minutes, after which the extent of ingestion was assessed microscopically.
To determine the opsonic activity of patient serum, control PMNLs were used and the capacity of the patient PMNLs to ingest C. albicans was determined in the presence of control serum.

Nitroblue tetrazolium (NBT) reduction. Tests of resting and stimulated (semiquantitative) NBT reduction were performed according to the methods of Sher et al. The percentage of NBT (reduced) containing PMNLs was evaluated. For the resting test the normal result is <10% positive PMNLs and for the stimulated test >90%.

Hexose monophosphate shunt (HMS) activity. For these studies pure neutrophils were used. Resting and postphagocytic HMS activity was determined by a modification1 of accepted procedures by measuring the extent of release of radiolabelled carbon dioxide (14CO2) from glucose radiolabelled in the C-1 position (o-glucose 14C; New England Nuclear, Boston, Mass., USA) present in the cell-suspending medium. In resting systems C. albicans was absent and in stimulated systems opsonized C. albicans was present in a 5:1 ratio with PMNLs.

MPO-mediated protein oxidation. Determination of resting and postphagocytic iodination of C. albicans was performed according to the method of Root and Stossel. All assays were done in duplicate and contained 1 x 106 PMNLs, 0.1 ml autologous serum, 0.1 ml 125I (sodium iodate; New England Nuclear) solution in phosphate-buffered saline (PBS) (0.6 µCi/ml). The final volume was made up to 1 ml with the PBS and the tubes rotated on a turntable at 37°C for 1 hour, after which the amount of 125I incorporated into acid-precipitable protein was assessed. To determine resting activity PBS was substituted for C. albicans.

Lymphocyte transformation. Blood for studies of lymphocyte function was defibrinated and fractionated by density gradient centrifugation (Ficoll : sodium metrizoate) at 400 g for 25 minutes. The mononuclear cell layer was removed and washed twice in medium RPMI 1640 (Grand Island Biological Co.), pH 7.2, supplemented with Heps, RPMI 2 g/l and 10% autologous heat-inactivated serum. The cell suspension was adjusted to 4 x 105 mononuclear cells/ml. Aliquots of 50 µl (2 x 106 cells) of this suspension were placed in wells of 6.0 mm Linbro tissue culture plates (Flow Laboratories, Inglewood, Calif., USA) together with 100 µl of serum-supplemented RPMI. The mitogens used in this study were phytohaemagglutinin (PHA) (Wellcome Reagents, Beckenham, UK) and concanavalin A (con A) (Sigma) at concentrations of 25 µg/ml and 50 µg/ml. Mitogens were added to triplicate wells in 20 µl volumes and unstimulated controls received 20 µl of RPMI. The plates were mixed and incubated for 48 hours in a humidified atmosphere of 3% CO2 in air, after which 20 µl of tritiated thymidine (3H-T; thymidine-methyl-3H; New England Nuclear) containing 0.2 µCi was added to each well and the plates incubated for a further 18 hours. Harvesting was performed using a multiple automated harvester (Mash II; Microbiological Associates, Bethesda, Md, USA). Incorporation of 3H-T was assessed in a liquid scintillation spectrophotometer.

Serological investigations

Immunoglobulins and complement components. The serum IgG, IgA and IgM levels were determined by radial immunodiffusion using commercial plates and standards (Behring Institute, Mannheim, West Germany). Serum C3 and C4 complement levels and salivary IgA levels were assayed by rocket immunoelectrophoresis. Agarose gel plates (20 x 10 cm) were employed, accommodating wells 32 x 2.5 mm in diameter. Serum samples were in 20 dilutions for both C3 and C4 estimations and applied in 5 µl volumes to each well. Dilutions of the appropriate serum standard were run with each plate.

Antiser to human C3 and C4 and IgA were obtained commercially (Behring). Calibration curves were constructed by appropriate dilutions of standard sera. Electrophoresis was performed at 10 V for 3 hours. Results of immunoglobulin and complement estimations are expressed as mg/dl and mg/l respectively. Serum IgE levels were measured by radio-immunoassay (Phadebas IgE test; Pharmacia Diagnostics, Uppsala, Sweden) and results expressed as IU/ml; the upper limit of normal is taken as 150 IU/ml. Serum C-reactive protein (CRP) levels were measured by a semiquantitative latex agglutination procedure, the normal value being < 13.2 µg/ml. Serum RF levels were measured by the Rose-Heller indirect haemagglutination test and results expressed as the reciprocal of the titre.

Results

Calculation and comparison of results. The results shown in Tables I and II are expressed as mean and standard error. For each test the values obtained before propranolol therapy have been compared with the corresponding values after 3 months by Student's t test.

Neutrophil studies. Results before and after 3 months of propranolol therapy are shown in Table I. No abnormality of PMNL phagocytosis (ingestion and opsonization) or postphagocytic metabolic activity was observed. No abnormality of postphagocytic metabolic activity was detected in any patient and no alteration of these functions was found following propranolol therapy. Two patients had depressed neutrophil chemotaxis to normal and autologous EAS which became normal following propranolol therapy. However, there was no significant difference in migratory values before and after treatment for the group (Table I).

Lymphocyte transformation. Significant stimulation of lymphocyte transformation to both mitogens was observed in 8 of the 10 patients following the administration of propranolol (Fig. 1). The P values for 25 µg PHA, 50 µg PHA, 25 µg con A

Fig. 1. The in vitro responsiveness of peripheral blood lymphocytes from patients with RA to the mitogens PHA and con A at concentrations of 25 µg/ml and 50 µg/ml, before and after 3 months of propranolol therapy.
TABLE I. NEUTROPHIL CHEMOTAXIS, RESTING AND STIMULATED NBT REDUCTION, HEXOSE MONOPHOSPHATE SHUNT ACTIVITY AND MYELOPEROXIDASE-MEDIATED PROTEIN IODINATION BEFORE AND AFTER 3 MONTHS OF PROPRANOLOL THERAPY

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opsonization (%)</td>
<td>93.0 ±1.1†</td>
<td>94.2 ±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Ingestion (%)</td>
<td>94.2 ±0.6</td>
<td>93.2 ±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>NBT reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (%)</td>
<td>7.4 ±2.6</td>
<td>6.4 ±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Stimulated (%)</td>
<td>92.3 ±2.5</td>
<td>94.2 ±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>HMI activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (%)</td>
<td>2507 ±293</td>
<td>2206 ±415</td>
<td>NS</td>
</tr>
<tr>
<td>Postphagocytic (cpm)</td>
<td>19473 ±190</td>
<td>20428 ±2120</td>
<td>NS</td>
</tr>
<tr>
<td>MPO iodination of protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting (cpm)</td>
<td>5806 ±1150</td>
<td>4609 ±987</td>
<td>NS</td>
</tr>
<tr>
<td>Stimulated (cpm)</td>
<td>17134 ±1231</td>
<td>18219 ±1301</td>
<td>NS</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control EAS (cells/HPF)</td>
<td>208 ±26</td>
<td>221 ±32</td>
<td>NS</td>
</tr>
<tr>
<td>Autologous EAS (cells/HPF)</td>
<td>206 ±31</td>
<td>187 ±17</td>
<td>NS</td>
</tr>
</tbody>
</table>

* As determined by Student's \( t \) test.
† Results expressed as mean value for 10 patients with standard error.
\( cpm = \) counts per minute; cells/HPF = cells per high-power field.

TABLE II. SERUM IMMUNOGLOBULIN, RF, CRP, C3 AND C4 AND THC LEVELS BEFORE AND AFTER 3 MONTHS OF PROPRANOLOL THERAPY

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/dl)</td>
<td>17.3 ±2.3†</td>
<td>14.9 ±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>3.1 ±0.4</td>
<td>2.8 ±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>1.7 ±0.2</td>
<td>1.5 ±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>IgE (IU/ml)</td>
<td>550 ±101</td>
<td>416 ±79</td>
<td>NS</td>
</tr>
<tr>
<td>C3 (mg/l)</td>
<td>70 ±5.4</td>
<td>73 ±6.2</td>
<td>NS</td>
</tr>
<tr>
<td>C4 (mg/l)</td>
<td>44 ±3.2</td>
<td>42 ±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>THC (CH(_{50}) units)</td>
<td>191 ±11.6</td>
<td>202 ±12.1</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (( \mu )g/ml)</td>
<td>72 ±14.1</td>
<td>80 ±16.2</td>
<td>NS</td>
</tr>
<tr>
<td>RF ( ^&lt; )</td>
<td>84 ±25.6</td>
<td>246 ±131.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

* As determined by Student's \( t \) test.
† Results as mean and standard error.
‡ Results as reciprocal of the titre.
THC = total haemolytic complement.

and 50 \( \mu \)g con A were \( P < 0.01, P < 0.05, P > 0.01 \) and \( P < 0.01 \) respectively.

Serological studies. No significant differences were found in the serum IgG, IgA, IgM, C3, C4, total haemolytic complement, C-reactive protein and RF levels before and after treatment with propranolol (Table II). Nine of the 10 patients had elevated serum IgE levels, which were reduced in 5 patients following propranolol; the difference was not significant, however.

Patients. The clinical data were insufficient for statistical evaluation and therefore no final conclusion can be drawn about the efficacy of propranolol per se. In summary: patient 1 improved during the 3-month treatment period but became worse after this, although propranolol was continued in this particular case; patient 2 experienced no change during or after the trial period; patient 3 improved steadily throughout, and remission was maintained after the withdrawal of propranolol; patient 4 remained unchanged during and after the trial; patient 5 improved initially, but experienced a severe relapse during the final month and subsequently died from 'malignant' rheumatoid disease; patient 6 improved during and maintained this state after the trial period; patient 7 improved over the 3 months and deteriorated on withdrawal of propranolol; patient 8 deteriorated steadily; patient 9 came into remission during the trial; and patient 10 deteriorated while on propranolol.

Discussion

This study has shown that the depressed \( \text{in vitro} \) responses of lymphocytes from patients with rheumatoid arthritis are increased following the addition of propranolol to standard therapy. However, the changes in lymphocyte function were not accompanied by significant alterations of neutrophil function or serum RF, CRP and IgE levels. Improved responsiveness to mitogens does not therefore appear to be related to disease activity.

Although 2 of the 10 patients had low neutrophil chemotaxis, the mean value for the group as a whole was above the lower limit of normal for this laboratory. This is contrary to the observation of Mowat and Baum, who described reduced neutrophil motility in such patients. We have since assessed the chemotactic responsiveness of neutrophils from 2 untreated patients with RA and found normal migratory responses. Neutrophil motility was not significantly increased after 3 months of propranolol therapy, although previous reports have indicated that this
function is stimulated in vitro and in vivo by propranolol. Correction of defective motility, however, was observed in the 2 patients with reduced responsiveness. Neutrophil phagocytic and postphagocytic metabolic activities were not detectably affected by propranolol.

Nine of the patients investigated in this study had elevated serum IgE levels, a finding which has been described previously. These 9 patients were also RF-positive. The patient who was seronegative for RF had a normal serum IgE value. Selectively raised IgE levels, or hyperimmunoglobulinaemia E, can be associated with secondary defects of neutrophil motility and lymphocyte transformation. Interestingly, in this study the 2 patients with depressed neutrophil chemotaxis had the highest serum IgE levels. The 1 patient who was seronegative for RF and had a normal IgE value also had normal chemotactic responsiveness and lymphocyte transformation on initial investigation. However, considerable research is required to determine the significance of raised serum IgE levels in patients with RA. It is possible that false-positive results occur in patients with RA when the Phadebas technique for IgE determinations is used.

Kaplan et al. have recently reported clinical improvement in 10 of 11 patients with RA following propranolol therapy. However, optimal effects were observed with doses of 200 - 640 mg/d. No alterations in the humoral parameters of disease activity were observed in the subjects, which is in agreement with this study. These authors chose to terminate their study because of the high proportion of patients (8 out of 10) whose RA worsened slowly after propranolol therapy had been discontinued, and the fibrosing disorders which may be associated with prolonged use of the drug.

Although we observed clinical improvement (which may be unrelated to propranolol therapy) in 4 out of 10 patients who received propranolol 120 mg/d, we have decided not to undertake further clinical studies, since the drug seems to be of doubtful value in the treatment of RA.

REFERENCES