Practolol and ocular toxicity

Antibodies in serum and tears

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The use of practolol, a β -adrenergic receptor blocking agent, in the management of cardiac dysrhythmia and ischaemic heart disease, is associated in a few patients with ocular disturbance (Wright, 1974, 1975; Rahi, Chapman, Garner, and Wright, 1976). Essentially this presents as a painful dry eye with subconjunctival fibrosis and occasional corneal ulceration. The eye lesions are usually associated with skin rash and both are linked with circulating antinuclear factors and antibodies which attach to the membranes of squamous epithelia (Wright, 1975; Rahi and others, 1976; Amos, Brigden, and McKerron, 1975). We here report our antibody findings in the sera and tears of affected patients.

Patients and methods

Twenty-two patients who had developed lesions or symptoms in the outer eye while receiving practolol (Eraldin) were investigated and allocated to one of two groups according to the severity of their condition. Group A consisted of 14 patients whose complaint of a hot, gritty sensation in the eyes was associated with reduced tear secretion and an abnormal conjunctival epithelium as evidenced by positive rose bengal staining. The eight patients in Group B had symptoms but they had few objective signs, or none, of ocular disease. Tear samples were collected either in capillary tubes or on triangular sponges from 15 patients and from 20 healthy individuals. Blood specimens were also obtained from each patient and from 100 control subjects.

Levels of serum immunoglobulins G, A, and M were estimated using a radial immunodiffusion technique (Mancini, Carbonara, and Heremans, 1965). IgM-rheumatoid factor and antithyroid antibodies were demonstrated using diagnostic kits supplied by Burroughs-Wellcome Ltd, while antinuclear and other autoantibodies were identified by standard indirect immunofluorescent techniques at an initial serum dilution of 1:10. Immunofluorescent specimens were examined using a Zeiss epifluorescence microscope with an FITC interference filter. The incidence of antibodies in patients and control subjects was compared using the χ^2 test.

Tear immunoglobulins, lysozyme, transferrin, and albumin were demonstrated by immunoelectrophoresis

in veronal buffer (pH 8.6, $I = 0.075$) at a constant current using polyvalent antihuman sera and antisera specific for IgG, IgM, IgA, and the secretory component of IgA. Lysozyme formed a characteristic non-immune precipitate during electrophoresis, the nature of which was confirmed in a few samples by elution and by testing against antilysozyme serum on Ouchterlony plates. In an attempt to quantify the protein levels the immunoelectrophoretic precipitin lines were scored according to their length, intensity, and distance from the antibody-containing trough. Precipitin lines recognizable only after staining with nigrosine were counted as +, while those seen in unstained preparations were scored as either ++ or ++++. In a separate study of the IgA content of tears it was found that + represented anti-

Table I Serum immunoglobulins and autoantibodies in control subjects and in patients with practolol-induced ocular disease

Category and no. of patients	Healthy subjects (100)	Group A (14)	Group B (8)
IgG (IU/ml)			
mean	121	137	123
range	70-282	98-220	86-163
IgA (IU/ml)			
mean	125	154	118
range	64-300	80-240	78-210
IgM (IU/ml)			
mean	151	142	129
range	67-300	96-269	100-201
<i>Autoantibodies (per cent positive)</i>			
Antinuclear antibody	15	64	25
Antinucleolar antibody	2	Nil	Nil
Smooth muscle antibody	16	28	13
Antibody to gastric parietal cells	3	Nil	Nil
Antireticulin antibody	2	Nil	Nil
Rheumatoid factor (rheumatoid test)	9	Nil	Nil
Antithyroglobulin antibody (dilution 1:25)	12	14	12

FIG. 1 Cryostat sections of rat liver pre-incubated with patient's serum and treated with fluorescein-conjugated antihuman immunoglobulin. Nuclear staining is of three types; (a) speckled ($\times 470$), (b) membranous ($\times 750$), and (c) diffuse ($\times 750$). Polygonal staining pattern at the cell periphery in (c) is due to presence of antibody to smooth muscle protein

FIG. 2 Cryostat section of rat stomach pre-incubated with patient's serum and treated with labelled antihuman IgG showing bright fluorescence in muscularis mucosae and vascular medial tunic. $\times 470$

body concentrations of less than 5 mg/100 ml, ++ approximated to 10 mg/100 ml, and +++ corresponded to a concentration of about 20 mg antibody/100 ml.

Results

The most striking serological finding (Table I) was the increased incidence of antinuclear antibodies in the more severely affected patients ($P < 0.001$), the pattern of immunofluorescence including diffuse, speckled, and membranous forms (Figs 1a, b, c). A rather less-distinct increase in incidence of antibody to smooth muscle was also observed in this group ($P < 0.05$) (Figs 1c and 2). Levels of immunoglobulins G, A, and M and other autoantibodies were not significantly different from those measured in control subjects. The only appreciable abnormality in the immunoelectrophoresis of tears in the patients was a reduction of secretory IgA, and this was found more frequently in the severely affected individuals (Table II).

Table II Immunoelectrophoresis of tears in healthy individuals and in patients with practolol-induced ocular disease

Group	No. tested	Total IgA	Secretory IgA	IgG	Transferrin	Albumin	Lysozyme
Healthy subjects	20	+++	++	+	+	+	+
Group A	7 (+1*)	+++	- in 6 + in 2	+	+	+	+
Group B	8	+++	+++ in 5 ± in 2 - in 1	+	+	+	+

*In one patient involvement was unilateral only: secretory IgA was absent from the tears of the affected eye but present in the contralateral eye

Discussion

The pathogenesis of the ocular and extraocular disturbances which can complicate practolol administration in certain individuals is obscure. IgG antibodies with an affinity for the intercellular region of squamous epithelium are usually, if not invariably, found. They are not obviously associated with structural damage to the cells, since they can be demonstrated in areas of healthy skin from patients with rashes involving other sites and do not seem to affect the desmosomes of corneal epithelium examined electron microscopically (Rahi and others, 1976). Moreover, although fixation of complement in the epidermis has been reported (Felix, Ive, and Dahl, 1974), we have not been able to produce convincing proof of such a reaction in ocular tissues (Rahi and others, 1976) and, since there is little to suggest an IgE-mediated response (Assem and Banks, 1973), there would seem to be scant evidence that the antibodies which are located in the epithelium have any cytotoxic effect.

The presence of antinuclear antibodies in a significant proportion of the patients we examined, particularly the more severely affected, invites comparison with certain drug-induced syndromes which resemble systemic lupus erythematosus. In such cases it has been suggested that the drug (procainamide, hydralazine, etc.) is metabolized in a way which causes it to react with nucleoprotein so that the nucleoprotein becomes autoantigenic (Blomgren and Vaughan, 1968; Tan, 1968). So far as practolol toxicity is concerned the extent to which antinuclear antibody produces tissue damage is unknown, although there is one report (Raftery and Denman, 1973) of membranous glomerular lesions which might be construed as evidence of a harmful deposition of nucleoprotein-antinuclear antibody complex.

In spontaneous autoimmune disorders, especially those which are organ-specific, it is common to find antibodies to a variety of tissues in addition to the target organ but, apart from a marginal increase in smooth muscle antibodies in the more severely

affected patients, this was not a feature of the practolol associated disease.

The absence or reduced amount of secretory IgA in the tears is almost certainly a measure of injury to the lacrimal gland, since the presence of apparently normal amounts of unconjugated IgA showed that there was no defect in antibody synthesis. This finding could be useful in assessing the degree of ocular involvement in patients at risk. Absence of the secretory component could, theoretically, reduce the capacity of IgA to combat infection in the outer eye but there is little to suggest that this was of practical importance and it is to be noted that the secretion of other antimicrobial factors, such as transferrin and lysozyme, was not noticeably impaired. Whether the parenchymal damage in the lacrimal gland is directly produced by the drug or its metabolites or if it is related to immunological activity is not known. Evidence that practolol may accumulate innocuously in lacrimal tissue of laboratory animals is difficult to evaluate since the ocular side-effects of the drug appear to be confined to man. Possibly the susceptibility to ocular disturbance is related to peculiarities in metabolism of the drug in a few individuals, so altering both nucleoprotein and other tissue antigens and rendering them autoantigenic and, at the same time, specifically damaging lacrimal gland epithelium.

Summary

Serological studies in 22 patients presenting with ocular disease attributable to dosage with the β -blocking agent practolol revealed a raised incidence of antinuclear antibodies. There was also a marginal increase in the incidence of antibodies to smooth muscle in the more severely affected individuals but the incidence of other autoantibodies and levels of IgG, IgA, and IgM were within normal limits. Semi-quantitative analysis of tears from 14 of the patients showed absence or near absence in the more severely affected patients of secretory IgA, which is indicative of damage to the lacrimal gland. Other immunological parameters in the tears were normal.

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