Mode of Action of Antirheumatic Drugs

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British Medical Journal

Summary

The concentrations of free and protein-bound L-tryptophan were measured in sera from normal subjects, patients with rheumatoid arthritis, pregnant women, and patients with jaundice. In the patients with rheumatoid arthritis receiving treatment with one or more antirheumatic drugs the percentage of the amino-acid bound to the circulating proteins was significantly depressed and in one patient returned to normal when therapy was stopped. Pregnancy and jaundice were also associated with raised free tryptophan and decreased bound tryptophan concentrations and bilirubin displaced the amino-acid from its binding sites on human serum proteins in vitro. It is suggested the behaviour of tryptophan mimics that of certain peptides which protect susceptible tissues against chronic inflammatory insults.

Introduction

Several attempts have been made to provide a common mechanism for the drugs used in the treatment of the rheumatic diseases. The more prominent include stimulation of the anterior pituitary and adrenal cortical axis; an interference with the biosynthesis, release, or action of suspected mediators of inflammation; uncoupling of oxidative phosphorylation reactions at the cellular level; or a stabilizing action on lysosomal membranes thus preventing the release of hydrolase enzymes which degrade joint cartilage. None of the suggested mechanisms adequately explains the clinical anti-inflammatory actions of the commonly used antirheumatic remedies (Whitehouse, 1965; Smith, 1966; Skidmore and Trnavsky, 1967; Spector and Willoughby, 1968; Collier, 1969).

All the antirheumatic drugs bind to circulating proteins and only a relatively small fraction exists in the free (non-protein-bound) form. Toxicity, rather than clinical effectiveness, is associated with an accumulation of the free drug (Dawkins and Smith, 1971). When the drugs bind to human serum proteins in vitro they displace other small molecules, including L-tryptophan and dipeptides (McArthur *et al.*, 1971). In the present paper we report that the displacement of tryptophan also occurs in patients with rheumatoid arthritis receiving therapy with a combination of antirheumatic drugs and in two conditions, pregnancy and jaundice, which are associated with an increased incidence of remissions in rheumatoid arthritis (Hench, 1949). It is suggested that the antirheumatic drugs act by displacing peptides from their binding sites to human serum proteins. The free forms of the peptides exert a protective action against chronic inflammatory processes.

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Subjects and Methods

Twenty-two patients aged 40-70 years with either classical or definite rheumatoid arthritis according to the criteria of the American Rheumatism Association (Ropes *et al.*, 1959) were studied. They comprised 19 outpatients (8 men and 11 women) and three inpatients (one man and two women). All were receiving therapy with one or more of the following drugs: aspirin, phenylbutazone, indomethacin, prednisolone, chloroquine, and gold salts. Ten pregnant women aged 21-33 and of 22-36 weeks' gestation and four women aged 47-66 with jaundice (two infectious hepatitis, two obstructive) were also investigated. Corresponding control groups were: six healthy men aged 36-53, six healthy women aged 38-53, six healthy women aged 24-31, and five women aged 49-65 who were in surgical wards (two preoperative and three 7-10 days postoperative for cholocystectomy, mastectomy, and arterial grafting). None of the healthy volunteers had taken any analgesic or anti-inflammatory drugs for at least 10 days before the experiment and none of the healthy women aged 24-31 were receiving contraceptive preparations. The jaundiced and surgical inpatients had been given the occasional barbiturate during the previous seven days but had not received any steroid therapy.

Blood samples (20 ml) were obtained by venepuncture into dry sterile containers. The total tryptophan concentrations were measured in the separated serum by the method of Hess and Udenfriend (1959), which was also used to determine the free (non-protein-bound) tryptophan concentrations in the serum ultrafiltrates obtained by centrifugation with Visking dialysis tubing (Goldstein, 1949).

Results

RHEUMATOID ARTHRITIS

The results for the outpatients with rheumatoid arthritis and for the corresponding control groups of normal subjects are given in Table I. Both groups of patients showed a significant reduction in the concentrations of total tryptophan and in the extent to which the amino-acid was bound to protein. The women also showed increased concentrations of free tryptophan but there was no difference between the two groups of normal subjects. In other experiments we found that there was no effect of age because sera from groups of men and women aged 20-35 gave values for free and total tryptophan concentrations identical with those from the older control groups in Table I. Despite the small number of patients involved, similar results were obtained with the group of rheumatoid arthritic inpatients when the results were compared with the surgical inpatients (Table II). One man aged 70, in hospital with rheumatoid arthritis and treated with a combination of aspirin, phenylbutazone, and indomethacin, was taken off therapy for four days. The results in Table III show the serum values for free and total tryptophan during treatment, on the fourth day after it had been stopped, and seven days after resumption of therapy.

When treatment was stopped the total trytophan concentration increased almost threefold whereas the free tryptophan concentration rose by only 40%. The binding curve of L-tryptophan to normal human serum (McArthur and Dawkins, 1969) shows that when the total tryptophan concentration increases then the percentage of the amino-acid bound to protein decreases. In the present instance the reverse occurred. Withdrawal of the drugs

allowed more of the tryptophan to circulate in the protein-bound form despite a much greater total concentration of the amino-acid being present in the serum. When the therapy was resumed for a further period the total tryptophan concentration and the proportion bound to protein fell to the values observed during the initial period of treatment.

TABLE I—*Mean Serum Tryptophan Concentrations and Standard Deviation in Normal Subjects and in Ambulant Patients with Rheumatoid Arthritis*

Tryptophan Concentration (μg%ml)	Men		Women	
	Normal (6)	R.A. (8)	Normal (6)	R.A. (11)
Free	0·83 ± 0·26	0·97 ± 0·26	0·84 ± 0·10	1·20 ± 0·34*
Total	12·39 ± 1·61	8·64 ± 1·51*	11·43 ± 1·50	7·19 ± 2·26*
Bound to protein (%)	93·2 ± 2·3	88·7 ± 2·6*	92·6 ± 1·0	82·1 ± 5·9*

In this and the subsequent tables the figures in parentheses show numbers of subjects or patients.
*Significance of difference from normal $P < 0.02$.

TABLE II—*Serum Tryptophan Concentrations in Female Surgical and Rheumatoid Inpatients*

Tryptophan Concentration (μg/ml)	Surgical (5)	R.A. (2)
Free	0·82 ± 0·15	1·41 ± 0·47*
Total	10·74 ± 2·24	5·26 ± 1·41*
Bound to protein (%)	92·3 ± 1·2	71·1 ± 16·8*

*Significance of difference between the two groups $P < 0.05$.

TABLE III—*Serum Tryptophan Concentrations in a Man with Rheumatoid Arthritis before, during, and after Therapy was stopped*

Tryptophan Concentration (μg/ml)	During Treatment	4 Days after Stopping Treatment	7 Days after Resuming Treatment
Free	1·30	1·85	1·57
Total	5·33	13·04	4·46
Bound to protein (%)	75·5	85·8	64·7

JAUNDICE

The serum concentration of free tryptophan was increased and the percentage of the amino-acid bound to circulating proteins was depressed in the jaundiced patients compared with the values obtained in a corresponding group of surgical inpatients (Table IV). At the time of the estimations the serum bilirubin levels in the patients with either infectious hepatitis or obstructive jaundice were in the range 10-30 mg/100 ml. Further

TABLE IV—*Serum Tryptophan Concentrations in Female Surgical and Jaundiced Patients*

Tryptophan Concentration (μg/ml)	Surgical (5)	Jaundiced (4)
Free	0·82 ± 0·15	1·67 ± 0·81*
Total	10·74 ± 2·24	9·43 ± 1·18
Bound to protein (%)	92·3 ± 1·2	82·4 ± 8·2*

*Significance of difference between the two groups $P < 0.05$.

experiments were performed to determine if bilirubin interfered with tryptophan binding to human serum proteins in vitro, the techniques described by McArthur *et al.* (1971) being used. It was found that the bile pigment, at concentrations of 10 mg/100 ml and above, displaced the amino-acid from pooled normal serum.

PREGNANCY

In a group of pregnant women (22-36 weeks' gestation) it was found that the serum-free tryptophan concentration was increased and the percentage of the amino-acid bound to protein was decreased when the values were compared with those obtained from a group of normal healthy women of similar age (Table V).

TABLE V—*Serum Tryptophan Concentration in Normal and Pregnant Women*

Tryptophan Concentration (μg/ml)	Normal (6)	Pregnant (10)
Free	0·76 ± 0·09	0·94 ± 0·19*
Total	11·38 ± 1·19	10·14 ± 1·57
Bound to protein (%)	93·4 ± 0·7	90·7 ± 1·8*

*Significance of difference between the two groups $P < 0.05$.

Discussion

All the effective antirheumatic drugs bind extensively to circulating proteins in man. It has been assumed that the protein-bound fraction is pharmacologically inert and that only the free (non-protein-bound) form causes the clinical and other actions. Extensive studies with salicylate have established that in-vitro effects of the drug on cellular metabolism occur only at concentrations far in excess of the free levels found in the serum during therapy (Dawkins and Smith, 1971). The protein-bound rather than the free fraction must therefore be responsible for the clinical antirheumatic actions.

When the drugs bind to serum proteins they displace other small molecules from their binding sites. Among these is L-tryptophan, the only amino-acid bound in man (McMenamy and Oncley, 1958). It is displaced from its binding sites to human serum proteins by salicylate, phenylbutazone, indomethacin, prednisolone, chloroquine, and gold salts in vitro (McArthur and Dawkins, 1969; McArthur *et al.*, 1971) and by the administration of aspirin to normal subjects in vivo (Smith and Lakatos, 1971). The present results show that this also occurs in patients with rheumatoid arthritis receiving therapy with a combination of the commonly used antirheumatic drugs. In one patient the various serum tryptophan concentrations returned to normal during a period in which therapy was stopped. The amino-acid itself has not been found to possess anti-inflammatory activity except for one report (Davis *et al.*, 1968) that it inhibits the infiltration of leucocytes into areas of local inflammation in the rat. Its displacement by the drugs, however, may reflect that of other compounds which could exert a protective action against the effects of chronic inflammatory insults on susceptible tissues, such as joints. If this is so then the serum tryptophan concentrations should alter in a predictable manner in conditions associated with remissions in rheumatoid arthritis. The two most prominent examples are jaundice and pregnancy (Hench, 1949).

The present results show that both conditions, in non-rheumatoid subjects, cause statistically significant changes in the concentrations of free tryptophan and in the degree of binding of the amino-acid to the circulating protein which would be expected if less of the amino-acid was attached to the serum protein. These effects may be explained by a displacement of the amino-acid by steroids in pregnancy and by bilirubin in jaundice.

If a group of small molecules exists in the circulation and their free forms protect tissues against chronic inflammatory processes then they should resemble tryptophan in being partially bound to serum proteins and in being displaced by the antirheumatic drugs. A possible candidate is peptides containing several amino-acids which are known to exist in the serum in bound and free forms (Rubin *et al.*, 1963). It has been shown that some synthetic dipeptides, in particular L-phenylalanyl-L-

phenylalanine, bind strongly to human serum proteins in vitro and are displaced by all the commonly used antirheumatic drugs (McArthur *et al.*, 1971). In addition, there should be some difference in the degree of binding of such "protective" peptides to normal serum proteins and to the proteins in serum of patients with rheumatoid arthritis. It has been reported (Denko *et al.*, 1970) that in patients with the rheumatic diseases the circulating albumin possesses an abnormal amino-acid composition, and this would be expected to be associated with different binding affinities and capacities for small molecules compared with normal serum albumin.

It is therefore proposed that the clinically useful drugs used in the treatment of rheumatoid arthritis and the other rheumatic diseases act by displacing peptides from their binding sites to serum proteins. The free form of the peptides protects connective tissues from the effects of inflammatory insults. In the rheumatic diseases there is a relative deficiency in the concentrations of free protective peptides in the circulation.

In the present paper preliminary experimental results supporting this hypothesis are presented. There remain certain critical experiments which need to be performed. It must be shown that the drugs displace peptides from their binding to the plasma proteins of normal subjects and of patients with rheumatoid arthritis and similar diseases. The displaced peptides must be separated and identified. There should be differences between the free concentrations of some of these peptides in normal subjects and in patients with rheumatic diseases who have not received therapy with any of the antirheumatic drugs. Finally, among the peptides displaced by the drugs should be one or more compounds which exert a protective action against chronic inflammatory stimuli.

There are several important implications of the above hypothesis. If the role of peptides, in their free forms, is established and if they can be identified then a more direct form of therapy in the rheumatic diseases becomes available. Chemical synthesis could provide a large range of useful compounds. Early diagnosis of rheumatoid conditions could be facilitated by measuring the free concentrations either of these peptides or of other molecules which are displaced to a similar extent by the currently used drugs. More efficient control of therapy could be assessed by serial measurements of free fractions of the peptides. The screening of potential anti-inflammatory substances would be placed on a more rational basis if activity were related to ability to displace peptides from circulating proteins.

We wish to thank Sir Edward Muir, Mr. L. T. Cotton, Mr. J. L. Dawson, Mr. J. M. Brudenell, and Dr. Roger Williams for allowing us to study patients under their care, and the King's College Hospital and Medical School Research Committee for financial support.

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Thyrotoxicosis in the African: Clinical and Immunological Observations

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British Medical Journal, 1971, 2, 679–681

Summary

The clinical manifestations of thyrotoxicosis are described in 20 African patients with toxic diffuse goitre (Graves's disease) and five with toxic nodular goitre. Antibody to thyroglubulin was detected in the serum of one patient and antibody to thyroid microsomes in four patients. Round-cell infiltration of the thyroid gland was present in 27% of 30 African thyrotoxic patients and 73% of appropriately matched Caucasian patients. It is suggested that the low incidence of thyrotoxicosis in the African race is related to an inability to form thyroid autoantibodies.

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Introduction

Thyrotoxicosis is considered to be rare among the indigenous populations of Africa. During the past decade about 50 cases were recorded in the world literature. Trowell (1960) saw two African patients with thyrotoxicosis in 30 years of medical practice in East Africa. Gelfand (1962) reported the first Rhodesian African patient in whom he was certain of the diagnosis. Thyrotoxicosis is rare among the indigenous populations of South Africa (Dancaster, 1970), Uganda (Patel, 1962), and Nigeria (Taylor, 1968). In Kenya thyrotoxicosis was thought to be extremely rare until Wright (1967) described eight cases. Taylor (1968) and Dancaster (1970) suggested that the rarity of thyrotoxicosis in the African is an immunological phenomenon related to an inability of the African to produce autoimmune antibodies. This problem has not been adequately studied because of the small numbers of patients encountered by each observer, and the total lack of data on autoantibody formation in African thyrotoxic patients.

This paper is a report of 25 African patients with thyrotoxicosis in whom thyroid autoantibodies have been measured.