Pattern of Urinary Proteins and Peptides in Patients with Rheumatoid Arthritis Investigated with the Iso-Dalt Technique

Penelope M. S. Clark, Larry J. Kricka, and Thomas P. Whitehead

Characteristic differences in the pattern of urinary proteins and peptides have been found in patients with rheumatoid arthritis, compared with patterns from healthy controls. These differences have been demonstrated with a two-dimensional gel electrophoretic technique (Iso-Dalt) involving isoelectric focusing in the first dimension, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in the second dimension. Using simple photographic techniques, one can obtain a composite pattern of the individual protein-stained gels for each group. Comparison of the composite patterns from the rheumatoid arthritis group and the control group revealed several proteins in the urine of the rheumatoid arthritis patients not found in the control group. Two groupings of these proteins were identified: acidic, high-M₄ proteins and more basic, low-M₄ proteins.

Additional Keyphrases: two-dimensional gel electrophoresis - isoelectric focusing

Several proteins and peptides have been isolated from the urine of patients with rheumatoid arthritis. Two main groups have been studied, both of which are thought to mirror connective-tissue involvement: (a) hydroxyproline-containing peptides (1–5) and (b) the glycosaminoglycans (6, 7). Though peptiduria and proteinuria are found in this disease group in the absence of renal disease, these conditions may also be associated with changes in renal function. Thus it has been suggested (8) that 70% of patients with rheumatoid arthritis have evidence of renal involvement.

Many of the studies on the excretion of proteins and peptides in rheumatoid arthritis have involved specific assays for particular proteins or peptides, e.g., measurement of urinary hydroxyproline (total, free, and bound) (1–5) and kinins (9). Investigation of the range of urinary excretion of peptides or proteins in rheumatoid arthritis has been limited.

In recent years, techniques for the “mapping” of proteins have developed; of these, the Iso-Dalt technique offers high resolution (10–13). This technique involves isoelectric focusing in the presence of urea in the first dimension, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in the second dimension. Thus proteins and peptides are separated by differences in charge and molecular size.

The aim of our study has been to investigate the urinary excretion of peptides and proteins in patients with rheumatoid arthritis by using the Iso-Dalt two-dimensional electrophoretic technique.

Methods

Clinical Material

Uretines (24-h collections with azide as preservative) were obtained from eight apparently healthy controls, from 11
patients suffering from rheumatoid arthritis, and from one patient with leukemia. Those suffering from rheumatoid arthritis either were housebound, and contacted through their general practitioner, or had been admitted to hospital for assessment and treatment. Informed consent, detailed medical and drug histories, and where possible, results of any relevant investigations were obtained.

Samples were stored frozen at -4 °C until analyzed. Urine 24-h creatinine amounts were determined by the Jaffé reaction on a continuous-flow analyzer (AutoAnalyzer II; Technicon Instrument Corp., Tarrytown, NY 10591) (14). No urine was used for which the creatinine value exceeded the mean ±2 standard deviations for the relevant subject group (rheumatoid arthritis patients and healthy controls), to ensure that complete 24-h collections had been obtained.

We also analyzed, by the Iso-Dalt technique, urine (24-h collection) from the patient with leukemia and a serum C-reactive protein of 325 mg/L, to investigate the influence of increased amounts of serum acute-phase proteins in serum on urinary protein excretion. Because serum concentrations of acute-phase proteins are also increased in patients with rheumatoid arthritis (15), it was necessary to demonstrate that any differences detected between the rheumatoid arthritis group and the normal group were not due simply to increased amounts of acute-phase proteins.

We screened urines for protein, hemoglobin, ketones, glucose, and pH, using Labstix (Ames Co., Stoke Poges, Slough, U.K.) to eliminate patients with renal involvement or other pathologies.

Iso-Dalt

Urinesto be analyzed were first treated as follows: an aliquot (900 mL) was dialyzed against distilled water (4 L)
overnight at 4 °C by use of 18/32° i.d. Visking tubing (Gal-
lenkamp Co., Ltd., London EC2P 2ER, U.K.). The aliquot was
then concentrated by dialysis against polyethylene glycol 6000
(400 g/L; BDH, Poole, Dorset, U.K.). The Visking tubing was
sealed at one end and attached to a funnel at the other. The
funnel was then filled with the urine to be concentrated, and
the Visking tubing was filled slowly with urine and placed in
the polyethylene glycol solution. As water was removed from
the urine sample, the dialysis bag was continually refilled with
urine from the funnel. This was continued until the final
volume of the urine was 2 mL.

An aliquot (1 mL) of each of the dialyzed concentrated
urines was then mixed with the “Iso” sample preparation
solution and left to stand at room temperature for 20–30 min.
These were then analyzed by the Iso-Dalt method of Anderson
and Anderson (11–13). The “Iso” sample preparation solution
consisted of sodium dodecyl sulfate (20 g/L; Sigma Chemical
Co., Poole, Dorset, U.K.), mercaptoethanol (5 mL/L; BDH),
and glycerol (200 mL/L; BDH).

For the first-dimension electrofocusing we used Ampholines
(pH 3–10; LKB, Croydon, Surrey, U.K.). We added 30–50 μL
of sample to each tube, using a Hamilton syringe (three sam-
ple of each). The gels then ran at 400 V for 12–20 h, the
voltage being increased to 700 V for the last 1–2 h.

The second-dimension polyacrylamide gel electrophoresis
was on slab gels with linear polyacrylamide gradients (100–200
g/L) and with a stacking gel. The plates were run at 1–1.2 A
for 3–4 h and then stained with Coomassie Brilliant Blue so-
lution. After destaining, the gels were put into water about 30
min before photographing.

Photography

A Kodak Technical Pan film SO-115, 5 × 4 in. (cat. no. 152
4584; Kodak, Rochester, NY 14650) was used to photograph
each gel. From the negatives of each group we constructed a
composite negative.

Results

Composites of the individual photographs of the pattern
of peptides and proteins for each patient in the rheumatoid
arthritis group and for the apparently healthy control group
are shown in Figure 1. A diagrammatic representation of the
differences found when comparing the two composites is
shown in Figure 2. These differences were found in the acidic,
high relative molecular mass (Mr) region and the basic, low-Mr,
region. The percentage of rheumatoid patients showing these
differences in the acidic, high relative molecular mass (Mr)
regions, a, b, and c, were 64, 82, and 73%, respectively, and 18%
in the basic, low-Mr, region. d. Photographs of typical gels from
a rheumatoid patient, a rheumatoid patient excreting low-Mr,
proteins, and the leukemia patient are shown in Figure 3.

None of the urines gave an abnormal result on testing with
Labstix.

Discussion

Rheumatoid arthritis, an inflammatory disease of the joints,
affects 3 and 10% of the populations of the United Kingdom
and United States, respectively (16, 17). Despite the preva-
ience of this disease, the clinical chemistry laboratory offers
few effective tests for either the early diagnosis or the man-
agement of rheumatoid arthritis. Though connective-tissue-
like proteins have been demonstrated in the urine of patients
with rheumatoid arthritis, the presence of these proteins has
not been shown to be of diagnostic significance (1–7).

In most of the previous studies, separation techniques with
limited resolution have been used. In contrast, the Iso-Dalt
technique is a high-resolution method by which a large
number of proteins in a mixture can be separated and com-
pared. This enables areas of differences to be pinpointed for
further investigation by easier, more specific techniques. Al-
though this technique has been used for the analysis of urinary
proteins (18, 19), its application to the study of particular
diseases has been limited.

Comparison of the Iso-Dalt patterns of urinary proteins of
the two groups we studied has revealed the presence of a
number of proteins in both the acidic, high-Mr, and basic,
low-Mr, regions of the gels from the rheumatoid subjects, but
not in those from the healthy controls. The location of these
proteins on the Iso-Dalt gels was such that they were unlikely
to be artefactual differences arising from a misalignment of
the group’s composite patterns.

The proteins found in the urines of the rheumatoid subjects
were not present in the urine from a patient with a high
amount of serum C-reactive protein. This suggests that these differences in urinary proteins are unlikely to arise simply as a result of increased amounts of acute-phase proteins in serum.

A few of the rheumatoid subjects (18%) showed low-Mr proteins in their urine by this technique (Figure 3). Further work is necessary to assess whether this reflects an early stage of renal involvement in the disease process.

A major problem with the Iso-Dalt technique is the enormous amount of information produced and the subsequent difficulties in data handling. The photographic technique described in this paper for comparing Iso-Dalt is within the capabilities of conventional clinical laboratories, requiring no expensive scanning and computing facilities. On the other hand, this technique has the disadvantage that subtle comparisons of intensities of protein-stained gels cannot be undertaken.

This preliminary study has demonstrated the utility of the Iso-Dalt technique in the study of urinary proteins in patients with rheumatoid arthritis. Further developments in the analytical technique to reduce the time for analysis will greatly enhance its value as a clinical laboratory technique.

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References