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BREATH PENTANE EXCRETION AS A MARKER OF DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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Activated inflammatory cells are capable of stimulating lipid peroxidation. In 27 patients with rheumatoid arthritis, we measured the pulmonary excretion of pentane, a product released during lipid peroxidation. We found highly significant correlations between pentane excretion and both joint inflammation ($r = 0.88$, $p < 0.001$) and the erythrocyte sedimentation rate ($r = 0.80$, $p < 0.001$). Patients treated with gold compounds or D-penicillamine excreted diminished amounts of pentane. The data suggest that lipid peroxidation may be related in part to the mechanism of injury in rheumatoid arthritis.

KEY WORDS: Pentane, breath test, rheumatoid arthritis, lipid peroxidation.

A growing body of literature suggests an association between lipid peroxidation and rheumatoid arthritis.[1-3] This peroxidation process is presumed to be stimulated by the release of oxygen radicals from neutrophils, macrophages and other inflammatory cells.[4-6] Oxygen radicals degrade unsaturated fatty acids to form lipid hydroperoxides, which then decompose forming aldehydes and alkanes.[7] One aldehyde, malondialdehyde, has been found to be increased in the plasma of one half of the patients with rheumatoid arthritis.[1] However, the assay used to measure malondialdehyde is not specific for this product of peroxidation, and the assay is more suited for in vitro experiments.[8] Pentane is an alkane which is a decomposition product of omega-6 unsaturated fatty acids such as linoleic acid.[9] Excretion of pentane in the breath is thought to be a specific marker of lipid peroxidation *in vivo*. In order to establish further the role of lipid peroxidation in rheumatoid arthritis, we measured breath pentane concentrations in patients with rheumatoid arthritis, and also correlated the magnitude of pentane excretion with the severity of illness in the study subjects.

METHODS

We studied 27 consecutive patients with definite or classical rheumatoid arthritis based on the criteria of the American Rheumatism Association. Patients with other coexistent rheumatic diseases were excluded from the study. Activity of disease was quantified by assessing joint inflammation using Lansbury's articular index (AI)[10] and

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TABLE I
Patient Characteristics

Patient	Age	Race Ethnic Group	Sex	Morning Stiffness *min*	Hand Grip	Articular Index	Westergren ESR	NSAID	Low Dose Prednisone?†	Second Line Drug	Pentane ppb
A.M.	71	Caucasian	F	< 30	Good	0	10	Naproxen	No	Methotrexate	10.0
E.W.	61	Black	F	< 30	Good	0	15		Yes	Methotrexate	15.9
M.D.	48	Black	F	< 30	Good	0	16	Aspirin	No	Gold	25.2
A.S.	58	Hispanic	F	< 30	Good	0	17		Yes	Methtrexate	26.3
F.A.	61	Black	F	< 30	Good	0	18	Sulindac	No	Gold	28.0
M.G.	59	Black	F	< 30	Good	0	18	Sulindac	No	Gold	31.8
C.B.	59	Caucasian	F	< 30	Good	0	20	Salsalate	No	Methotrexate	8.5
B.G.	54	Caucasian	F	< 30	Good	4	10	Salsalate	No	Methotrexate	9.0
R.O.	58	Caucasian	F	50	Fair	12	25	Sulindac	No		30.9
F.D.	29	Hispanic	F	30	Fair	16	22	Naproxen	No		25.9
B.D.	60	Black	F	60	Fair	19	26	Indomethacin	No	Gold	31.5
L.I.	51	Caucasian	F	75	Fair	24	38	Aspirin	No	Penicillamine	56.9
A.W.	66	Caucasian	F	30	Fair	25	22	Naproxen	Yes	Gold	37.0
L.L.	57	Black	F	120	Fair	25	40	Diflunisal	No		35.7
M.H.	32	Hispanic	F	90	Fair	26	30	Sulindac	Yes		50.8
L.W.	42	Black	F	80	Fair	26	32	Naproxen	No	Gold	84.0
B.S.	50	Caucasian	F	75	Fair	28	35		Yes	Methotrexate	67.2
M.T.	35	Hispanic	F	75	Poor	30	36	Naproxen	No	Gold	84.5
B.F.	53	Caucasian	M	150	Poor	30	36	Naproxen	No		69.4
C.S.	69	Caucasian	M	120	Poor	32	34	Aspirin	No	Methotrexate	83.7
B.D.	40	Black	F	75	Poor	56	42	Sulindac	No	Hydroxychloroquine	63.1
F.D.	31	Hispanic	F	210	Poor	84	73	Aspirin	No		102.0
J.F.	57	Caucasian	M	180	Poor	88	46	Aspirin	Yes		265.7
F.D.	48	Black	F	180	Poor	88	61	Aspirin	No	Gold	116.7
J.R.	49	Black	F	N.D.	N.D.	97	64	Naproxen	No		230.0
W.M.	52	Caucasian	M	360	Poor	102	130	Aspirin	No		196.2
G.B.	52	Black	F	N.D.	N.D.	108	106	Aspirin	No		360.0

† ≤ 10 mg, per day
ND = not determined

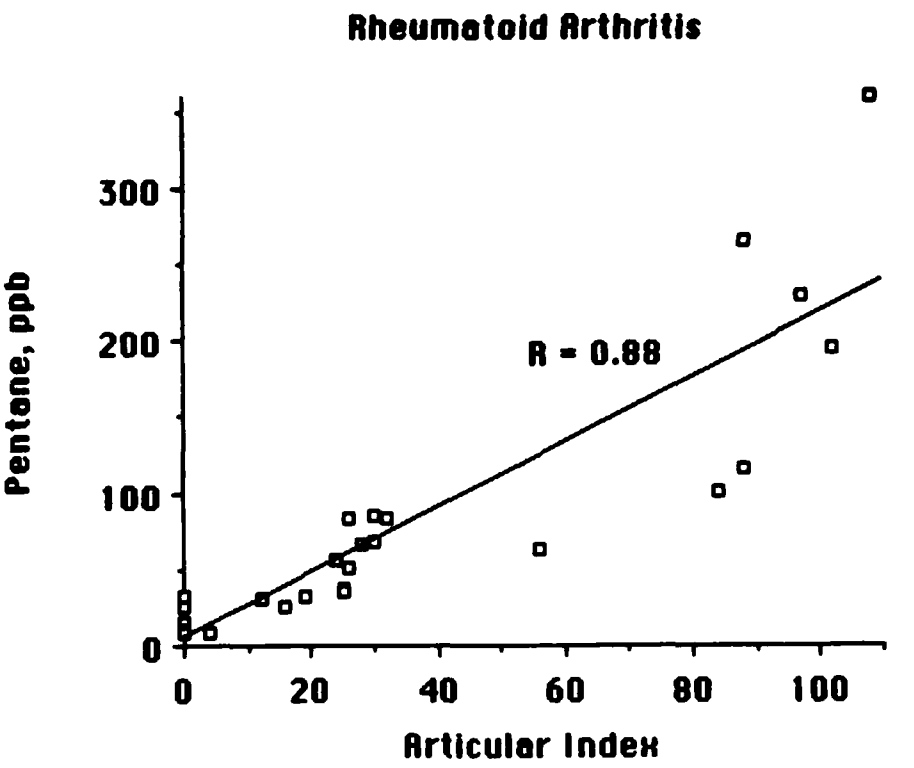

FIGURE 1 Relationship of pentane in expired air to articular index in unselected patients with rheumatoid arthritis.

Westergren sedimentation rate. Disease activity was assessed without knowledge of the pentane value. Duplicate breath samples were collected in the morning after an overnight fast using a modified Haldane-Prestley tube.[11] The breath samples were stored in 50 ml plastic syringes (Fortuna syringe, Aldrich Chemical Company, Milwaukee, WI)[12] and analyzed for pentane content within six hours of collection. Pentane analysis was conducted by gas chromatography (Model 6000, Varian Instruments, Sunnyvale, CA) using a 2 meter Chromasorb 102 column attached to a flame ionization detector.[12] Nitrogen was the carrier gas. The breath samples were injected into a gas sampling valve equipped with a 10 ml sampling loop. At injection, the sample was cold-trapped and then subjected to a multistep temperature program. This method of analysis was standardized[12] using commercially prepared gases (Alltech Associates, Ltd., Deerfield, Il.) over a range between 10 parts per billion (ppb) (0.44 nmole/litre) and 1045 ppb (43.5 nmole/litre). Statistical analysis was conducted by linear regression analysis.

RESULTS

We studied 27 patients (23 women and 4 men) with rheumatoid arthritis who ranged between 29 and 71 years of age. Table I shows the distribution of age, articular index,

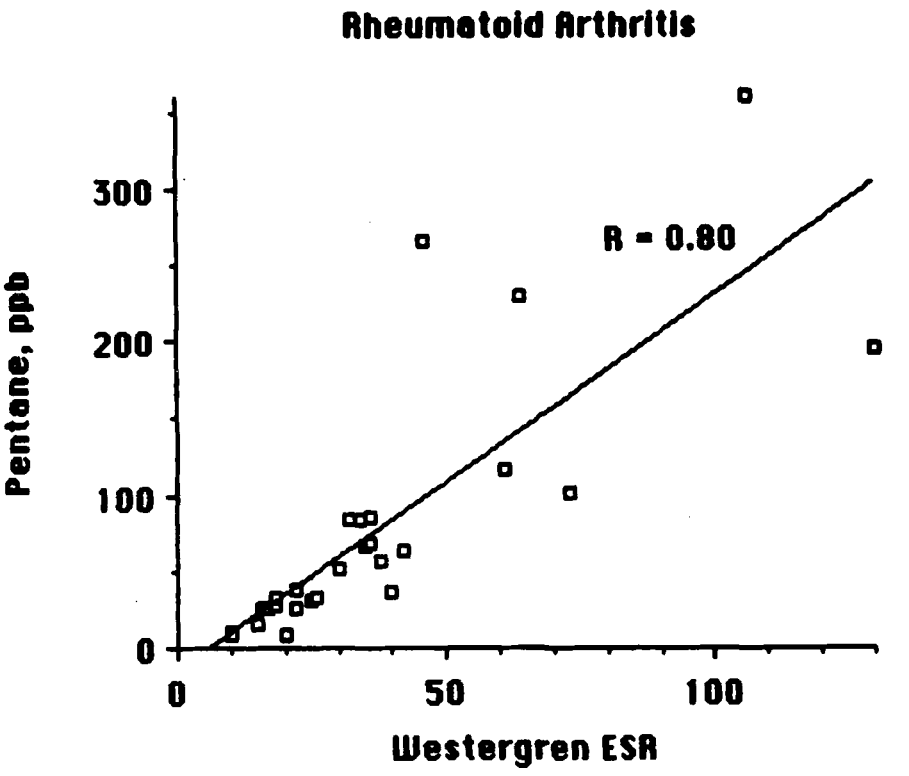

FIGURE 2 Relationship of pentane in expired air to Westergren sedimentation rate in unselected patients with rheumatoid arthritis.

sedimentation rate, and anti-arthritic therapy of these patients. We found a significant correlation between breath pentane excretion and articular index ($r = 0.88$, $p = < 0.001$) (Figure 1). We also found statistically significant correlations between pentane excretion and sedimentation rate ($r = 0.80$, $p < 0.001$) (Figure 2) and between articular index and sedimentation rate ($r = 0.89$, $p < 0.001$) (not shown). There was no correlation between pentane concentrations and patient age ($r = 0.12$). Patients receiving only non-steroidal anti-inflammatory drugs (2 patients also received low dose prednisone) had a correlation between pentane and articular index ($r = 0.89$) which was significantly different ($p < 0.005$) from that of those subjects receiving gold compounds (with low dose prednisone in one case) or D-penicillamine in addition to the NSAIDS ($r = 0.87$) (Figure 3). This difference between slopes persists ($p < 0.05$) even if the most severely ill patient treated with gold compounds is regarded to be an outlier and deleted from the computation.

DISCUSSION

We have demonstrated that pulmonary excretion of pentane is highly correlated with the activity of rheumatoid arthritis in 27 consecutive patients. Pentane excretion is not

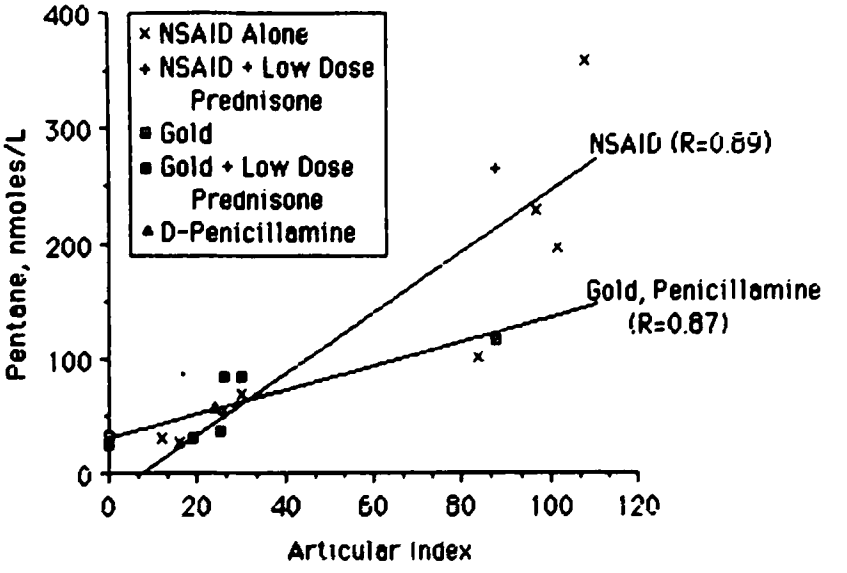

FIGURE 3 Relationship of pentane in expired air to articular index in patients with rheumatoid arthritis treated either with non-steroidal anti-inflammatory drugs (NSAID) or with gold compounds or D-Penicillamine. Two patients in the NSAID treatment group and one patient receiving parenteral gold compound also received low-dose (10 mg/da) prednisone.

affected by the duration of illness or age of the patient. This belief that pentane excretion is a marker of the severity of inflammation present in these patients is consistent with a previous report which correlated pentane excretion with chemically-induced inflammation in rats.[13] This opinion is also supported by previous reports of increased amounts of thiobarbituric acid (TBA)-reactive material (malondialdehyde) in the synovial fluid of some patients with rheumatoid arthritis.[3] However, not all TBA reactive material is a product of lipid peroxidation,[8] and hence the TBA assay appears to be neither sensitive nor specific for lipid peroxidation in patients with rheumatoid arthritis.

It is not known whether the peroxidation noted in patients with rheumatoid arthritis is due primarily to a failure of the protective antoxidant systems [14] (e.g. catalase, superoxide dismutase, ascorbic acid, vitamin E or glutathione) or to an excessive production of oxygen free radicals. The second option seems more likely, since stimulation of the large numbers of neutrophils located in the synovial fluid of patients with active rheumatoid arthritis is expected to result in enhanced oxygen radical generation.[4-6] It has been suggested that drugs such as D-penicillamine and gold compounds act in part by interfering with oxygen radical action.[15] In earlier studies we demonstrated that these drugs and related sulfhydryl drugs interfere with the oxidataive inactivation of proteinase inhibitors, a reaction which is oxygen radical mediated.[16] Our current data showing decreased pentane excretion in patients receiving these drugs further supports this position. Further definition of the mechanism of action for these drugs will require in vitro testing using markers of lipid peroxidation.

In summary, we have demonstrated a clinical correlation between the activity of disease in patients with rheumatoid arthritis and the pulmonary excretion of pentane.

Pentane is produced during lipid peroxidation, which may be partially responsible for the tissue damage seen in this disease.

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