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EXPIRED HYDROCARBONS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Pentane and isoprene concentrations were analyzed in single end-expiratory breath samples using gas chromatography. Breath analysis was performed in 15 patients with acute myocardial infarction, 15 patients with stable angina, and 15 healthy control subjects. The two patient groups were well matched for age, sex, smoking habits, hypertension and serum cholesterol levels. There was no significant difference in breath pentane concentration in the acute myocardial infarction group (0.29 ± 0.03 nmol/l) (mean ± SEM) compared to the group with stable angina (0.31 ± 0.03 nmol/l) or the control group (0.36 ± 0.04 nmol/l). However, breath isoprene concentration was higher ($p < 0.01$) in the acute myocardial infarction group (11.4 ± 1.2 nmol/l), compared to both the stable angina group (7.7 ± 0.5 nmol/l) and the control group (7.1 ± 1.0 nmol/l). There was no difference in either the pentane or isoprene concentrations between the control group and the group with stable angina. Since pentane is thought to be an index of lipid peroxidation, the results do not support the presence of enhanced lipid peroxidation in acute myocardial infarction in the absence of thrombolytic therapy or primary angioplasty. The mechanism responsible for isoprene elevation in acute myocardial infarction is unknown.

KEYWORDS: lipid peroxidation, free radicals, ischemia, pentane, isoprene, gas chromatography.

INTRODUCTION

Oxygen derived free radicals are produced by several different reactions in the body and are capable of damaging multiple cellular components. Increased free radical formation has been shown to occur in experimental animals during both ischemia and reperfusion.[1,2] Polyunsaturated fatty acids are particularly susceptible to free radical attack resulting in the formation of relatively stable lipid peroxides. Malondialdehyde is a major reaction product of lipid peroxidation and has been shown to be elevated in the plasma of patients with acute myocardial infarction following successful thrombolysis.[3,4]

In addition to malondialdehyde formation, peroxidation of lipids has also been shown to result in the formation of short-chain alkanes. Pentane is generated from peroxidation of w-6 polyunsaturated fatty acids and its assay in human breath is thought to represent a non-invasive index of lipid peroxidation.[5,6] Several studies have reported elevated breath pentane levels in disease states where there is inflammation or tissue degeneration.[7–11] Although pentane is present in expired air, the major hydrocarbon constituent of human breath is thought to be isoprene.[12–15] Since isoprene co-elutes with pentane on most chromatography columns,[13,14] many previous studies

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may have unknowingly reported pentane as the combination of isoprene and pentane. Elevated breath pentane levels have been reported in patients with acute myocardial infarction, however, the breath isoprene concentrations were not reported.[16] Thus, the purpose of the present study was to analyze both isoprene and pentane in patients with acute myocardial infarction.

MATERIALS AND METHODS

The study subjects included 15 patients with acute myocardial infarction, 15 patients with stable angina and 15 healthy controls. The patients with acute myocardial infarction were found to satisfy the World Health Organizations's 1983 criteria for the diagnosis of acute myocardial infarction.[17] Serum creatine kinase MB concentration was considered to be elevated when the fraction was 3% or greater. All breath samples were obtained in the coronary care unit before the patients received thrombolytic therapy or coronary angiography. The patient control group consisted of 15 patients with stable coronary artery disease diagnosed by coronary angiography. They were matched with the acute myocardial infarction group for age, sex, smoking status, diabetes and hypertension. The exclusion criteria were cardiac failure, cardiogenic shock, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty within the previous 3 months, liver disease, sepsis or acute inflammatory disease. The normal control group consisted of 15 age- and sex-matched, healthy, non-smoking volunteers.

We collected end-expiratory breath samples (125 ml) from all subjects using a polyvinylchloride tube (1.3 cm inside diameter) with a disposable mouth piece at one end and a one-way valve at the other. Breath samples were aspirated into air-tight glass syringes (60 ml), through a stopcock inserted just proximal to the one-way valve. Samples were collected in the morning at least three hours after the last meal and analyzed within two hours. All subjects were breathing room air for at least one hour prior to breath sampling. The pentane concentration in room air was found to be less than 0.05 nmol/l.

For the analyses of hydrocarbons in expired air, we used a gas chromatograph (Hewlett Packard 5890 series II) equipped with a gas sampling valve, a 10-ml sampling loop and a flame ionization detector. The sample loop was flushed with 40 ml of sample and manually pressurized to 800 mm Hg with the last 20 ml using a digital manometer (UM 2000/200, Netech Corp.). The hydrocarbons were eluted by passing the sample through a 25-m (0.53 mm diameter) Poraplot U capillary column (Chrompack). Helium was used as the carrier gas at a flow rate of 8 ml/min. The temperature of the sampling loop was 70°C, the injector temperature was 90°C and the detector temperature was 190°C. To minimize peak broadening from the large sample volume, the column temperature was held at 30°C for 2 minutes in order to concentrate the hydrocarbons at the head of the column. Thereafter, the column temperature was increased 40°C/min to 90°C, 5°C/min to 135°C and 50°C/min to a final temperature of 190°C.

Calibration curves were plotted using a commercially available reference gas mixture of hydrocarbons (C^2-C^6) with a concentration of 100 ppm (Alltech Associates) and appropriate dilutions. Calibration curves were plotted using five pentane concentrations; 0 ppb, 20 ppb (0.82 nmol/l), 50 ppb (2.04 mnol/l) 100 ppb (4.09 mnol/l) and 500 ppb (20.4 nmol/l). The concentration of isoprene was estimated from the pentane calibration curve.[15]

TABLE 1
Clinical characteristics of patients with acute myocardial infarction and stable angina.

	Acute Myocardial Infarction	Stable Angina
Age {mean(range)}	64 (45–87)	61 (38–84)
Male/female	14/1	13/2
Current smokers	40%	33%
Diabetes	33%	27%
Hypertension	47%	53%
Serum cholesterol (mmol/l)	5.73 ± 1.45	5.36 ± 1.46
Nitrates	100%	73%
Aspirin	80%	73%
Beta blockers	53%	53%
Calcium channel blockers	27%	40%
Anticoagulants	53%	27%

Chromatogram data acquisition, storage and peak area integration was done using a 386 PC and Peaksimple II software (SRI Instruments). Where possible the results are expressed as the mean ± SEM. One way analysis of variance was used to compare age, breath pentane and breath isoprene concentrations. If the ANOVA yielded a significant F value then individual group means were compared with a Student-Newman-Keuls test. A Fisher's exact test or Student's unpaired t-test were used to compare clinical characteristics in the two patient groups. All differences were considered to be significant when $p < 0.05$.

RESULTS

The clinical characteristics of patients with acute myocardial infarction and stable angina are compared in Table 1. There was no significant difference in the age, sex and major coronary risk factor profile (smoking, hypertension and diabetes), between patients with acute myocardial infarction and those with stable coronary heart disease. There was also no significant difference in the number of patients taking anti-anginal or antithrombotic medications. The mean age of the control group was 64 (range 42–78) and not significantly different from the two patient groups.

Electrocardiography of the acute myocardial infarction group showed 6 anterior and 9 inferior infarcts. The concentration of serum creatine kinase in the patients with acute myocardial infarction was 959 ± 209 U/l and the MB fraction was 63.6 ± 15.5%. The onset of chest pain before breath sampling was 17 ± 1.8 hours.

Figure 1 shows a chromatogram of a breath sample from a patient with acute myocardial infarction. The elution order of the organic components that were identified with reference standards was ethanol, pentane, isoprene and acetone. Acetone showed the greatest relative peak area followed by isoprene, ethanol and pentane. Table 2 shows the mean concentrations of pentane and isoprene in the three different subject groups. There was no significant difference in the pentane concentration in expired air in the group of patients with acute myocardial infarction compared to group with stable angina or the control group. The isoprene concentration in expired air was significantly elevated in the group with acute myocardial infarction compared to group with stable angina and the control group. There was no significant difference in breath

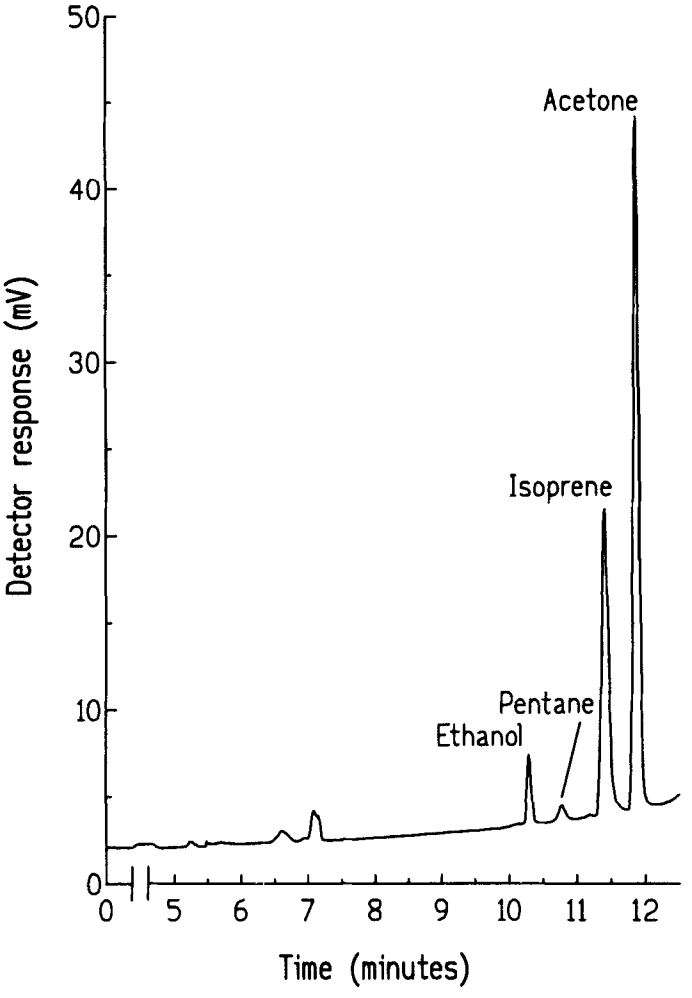

FIGURE 1 Gas chromatogram of an expired air sample from a patient with acute myocardial infarction. Retention time of ethanol, 10.3 min; pentane, 10.7 min; isoprene, 11.4 min; acetone, 11.9 min.

pentane and isoprene concentrations between the group with stable angina and the control group.

DISCUSSION

The results of this study indicate that isoprene in breath is elevated in acute myocardial infarction. Contrary to previously published work,[16] we did not find any increase in pentane concentration in the breath of patients with acute myocardial infarction. Weitz[16] reported an average pentane level of about 5 nmol/l in 10 patients with acute

TABLE 2
Effect of acute myocardial infarction on pentane and isoprene concentrations in end-expiratory air.

	Control	Acute Myocardial Infarction	Stable Angina
Pentane (nmol/L)	0.36 ± 0.04	0.29 ± 0.03	0.31 ± 0.03
Isoprene (nmol/L)	7.1 ± 1.0	11.4 ± 1.2*	7.7 ± 0.5

*$p < 0.01$ compared to stable angina and control

myocardial infarction compared to 2 nmol/l in a group of patients with chest pain and no infarction. Since these investigators did not report isoprene levels or document the separation of isoprene from pentane, it is conceivable that their pentane levels reflect a combination of isoprene and pentane. Indeed, the concentration of pentane in the breath of their control subjects (2 nmol/l) was much higher than the pentane concentrations that we observed or that have been reported by others using chromatographic techniques that separate pentane from isoprene.[13,14,18]

Several studies have reported that pentane levels in expired air from healthy subjects cannot be distinguished from pentane levels in room air.[6,14,18] In the present study, as well as a previous study,[15] we consistently observed a higher concentration of pentane in expired air compared to room air. Since there is no commonly accepted method for assaying pentane in human breath, it is difficult to compare the results of different studies. Our pentane levels are probably higher than the levels reported by others because we sampled end-expiratory breath rather than total breath. Furthermore, we minimized the possibility of pentane loss from the breath samples by the direct injection of the samples into a gas chromatograph.

Although both ischemia and reperfusion have been shown to increase the formation of free radicals in experimental animals, reperfusion is a much more effective stimulus for free radical formation.[1,2] Increased plasma levels of malondialdehyde have been demonstrated in patients with acute myocardial infarction following successful thrombolysis but not in those patients whose arteries remained occluded despite thrombolytic therapy.[3,4] Since malondialdehyde and pentane are both end products of lipid peroxidation, increased pentane formation would not be expected in acute myocardial infarction in the absence of spontaneous thrombolysis. Coronary arteriography has demonstrated spontaneous thrombolysis in only 28% of patients with acute myocardial infarction even when performed at the time of discharge.[19]

The mechanism responsible for the increase in isoprene in exhaled air in acute myocardial infarction is not clear. Earlier studies have suggested that the amount of isoprene excreted into breath is not influenced by diet, age, sex or fasting[20] but rather is elevated during sleep.[21,22] Isoprene formation is thought to be regulated in part by the same enzyme (3-hydroxy-3-methyl-glutaryl-CoA reductase) that regulates cholesterol synthesis.[23] Inhibition of cholesterol synthesis with lovastatin has been shown to cause a decrease in breath isoprene excretion.[22] There is also evidence that the synthesis of isoprene may be linked to the respiratory burst activity of neutrophils.[24] It is possible that activation of neutrophils in the ischemic myocardium may have been responsible for the increased formation of isoprene. It is also possible that isoprene is elevated in other inflammatory conditions that were previously found to be accompanied by enhanced pentane excretion.[7–11] Additional studies on the dynamics and mechanism of isoprene formation will be required to determine the precise mechanism responsible for the changes in isoprene in acute myocardial infarction.

References

1. J.L. Zweier, J.T. Flaherty and M.L. Weisfeldt (1987) Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proceedings of the, National Academy of Sciences of the United States of America*, **84,** 1404–1407.
2. R. Bolli, B.S. Patel, M.O. Jeroudi, E.K. Lai, P.B. McCay (1988) Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap alpha phenyl N-tert-butyl nitrone. *Journal of Clinical Investigation*, **82,** 476–485.
3. S.W. Davies, K. Ranjadayalan, D.G. Wickens, T.L. Dormandy and A.D. Timmis (1990) Lipid peroxidation associated with successful thrombolysis. *Lancet*, **335,** 741–743.
4. I.S. Young, J.A. Purvis, J.H. Lightbody, A.A. Adgey, and E.R. Trimble (1993) Lipid peroxidation and antioxidant status following thrombolytic therapy for acute myocardial infarction. *European Heart Journal*, **14,** 1027–1033.
5. K.N. Jeejeebhoy (1991) In vivo breath alkane as an index of lipid peroxidation. *Free Radicals in Biology & Medicine*, **10,** 191–193.
6. C.M. Kneepkens, C. Ferreira, G. Lepage and C.C. Roy (1992) The hydrocarbon breath test in the study of lipid peroxidation; principles and practice. *Clinical & Investigative Medicine*, **15,** 163–186.
7. S. Moscarella, G. Laffi, G. Buzzelli, R. Mazzanti, L Caramelli and P. Gentilini (1984) Expired hydrocarbons in patients with chronic liver disease. *Hepato-gastroenterology*, **31,** 60–63.
8. S. Humad, E. Zarling, M. Clapper and J.L. Skosey (1988) Breath pentane excretion as a marker of disease activity in rheumatoid arthritis. *Free Radical Research Communications*, **5,** 101–106.
9. P.K. Toshniwal and E.J. Zarling (1992) Evidence for increased lipid peroxidation in multiple sclerosis. *Neurochemical Research*, **17,** 205–207.
10. J. Kokoszka, R.L. Nelson, W.I. Swedler, J. Skosey and H. Abcarian (1993) Determination of inflammatory bowel disease activity by breath pentane analysis. *Diseases of the Colon & Rectum*, **36,** 597–601.
11. P.S. Sobotka, D.K. Gupta, D.M. Lansky, M.R. Costanzo and E.J. Zarling (1994) Breath pentane is a marker of acute allograft rejection. *Journal of Heart & Lung Transplantation.*
12. M. Phillips and J. Greenberg (1992) Ion-trap detection of volatile organic compounds in alveolar breath. *Clinical Chemistry*, **38,** 60–65.
13. D. Kohlmuller and W. Kochen (1993) Is n-pentane really an index of lipid peroxidation in humans and animals? A methodological reevaluation. *Analytical Biochemistry*, **210,** 268–276.
14. A. Cailleux and P. Allain (1993) Is pentane a normal constituent of human breath? *Free Radical Research Communications*, **18,** 323–327.
15. S. Mendis, P.A. Sobotka and D.E. Euler (1994). Pentane and isoprene in expired air in humans: gas chromatographic analysis of single breath. *Clinical Chemistry*, **40,** 1485–1488.
16. Z.W. Weitz, A.J. Birnbaum, P.A. Sobotka, E.J. Zarling and J.L. Skosey (1991) High breath pentane concentrations during acute myocardial infarction. *Lancet*, **337,** 933–935.
17. R.F. Gillum, S.P. Fortmann, R.J. Prineas and T.E. Kottke (1984) International diagnostic criteria for acute myocardial infarction and acute stroke. *American Heart Journal*, **108,** 150–158.
18. M. Phillips, J. Greenberg and M. Sabas (1994) Alveolar gradient of pentane in normal human breath. *Free Radical Research*, **20,** 333–337.
19. T.L. Schreiber, D.H. Miller, D.A. Silvasi, J.W. Moses, J.S. Borer (1986) Randomized double blind trial of intravenous streptokinase for acute myocardial infarction. *American Journal of Cardiology*, **58,** 47–52.
20. D. Gelmont, R.A. Stein and J.F. Mead (1981) Isoprene—the main hydrocarbon in human breath. *Biochemical Biophysical Research Communications*, **99,** 1456–1460.
21. A. Cailleux and P. Allain (1989) Isoprene and sleep. *Life Science*, **44,** 1877–1880.
22. B.G. Stone, T.J. Besse, W.C. Duane, C.D. Evans and E.G. DeMaster (1993) Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. *Lipids*, **28,** 705–708.
23. M.S. Brown and J.L. Goldstein (1980) Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *Journal of Lipid Research*, **21,** 505–517.
24. G.M. Bokoch and V. Prossnitz (1992) Isoprenoid metabolism is required for stimulation of the respiratory burst oxidase of HL-60 cells. *Clinical Investigation*, **89,** 402–408.

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