Thermochimica Acta, 185 (1991) 335–343
Elsevier Science Publishers B.V., Amsterdam

Thermoanalytical methods as a new approach to the study of atheromasic plaque

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(Received 17 December 1990)

Abstract

The final stage of the atherosclerotic lesion of vessels is an ulcerated plaque. A very interesting site of atheromasic plaque is in the epiaortic trunks, especially in the carotid vessels where occluding plaques can also be formed. Sometimes non-occluding plaques are potentially harmful due to their possible ulceration and embolisation. Using new techniques, such as Doppler analysis, etc., it is now possible to obtain accurate images reflecting the dimension, composition and structure of the plaques. At the same time, the possibility of removing the plaque by surgery means that it must first be established which plaques are at risk. Therefore, it is desirable to establish a relationship between the instrumental signals and the real risk by techniques which can provide information on the composition, structure and stability of the plaques and to correlate this information with the data from the Doppler analysis. Instrumental techniques such as thermal analysis, atomic absorption spectroscopy, ICP analysis and IR spectroscopy were used to carry out this study.

INTRODUCTION

Following the guidelines of the World Health Organization (WHO), atherosclerosis is identified as a series of "intima" modifications that induce local accumulation of lipids and blood elements (platelets, white cells, etc.), formation of fibrous tissue, calcification and morphological changes of vascular "media". The main lesions of atherosclerosis [1–3] consist of three progressive steps: the fat stria, fibrous plaque and complicated lesion phases. The fat stria phase is already present in pediatric patients, although here it is considered a reversible lesion constituted of macrophages and myocites full of cholesterol. Fibrous plaque is a cap of fibrous tissue covering proliferated elements of the "intima" and macrophages full of cholesterol (foam cells), and non-cellular substances such as lipids and elastic fibres. The final stage is the ulcerated plaque, containing cellular debris, and fibrinous and calcific materials. Fibrous plaques appear in the third stage, while atherosclerosis of the cerebral vessels develops later, its evolution being slower than in the aorta and in the coronaries.

0040-6031/91/$03.50

A very interesting site of atheromasic plaques is in the epiaortic trunks, especially in the carotid vessels where occluding plaques can be formed which block blood-flow to the brain. Sometimes, non-occluding plaques can also be very dangerous because of the possible occurrence of circulating emboli causing cerebral ictus and consequent pathologies. However, at present it is impossible to establish a reliable correlation between the degree of obstruction and the ensuing mortality risk, because the progression of the stenosis is also influenced by other factors.

Moreover, sometimes non-occluding plaques can be harmful due to their possible ulceration and embolisation.

The application of new techniques such as Ecodoppler of the epiaortic vessels, Transcranic Doppler, Ecocardiogram, etc., have substantially modified the methodological approach to cerebrovascular pathology. It is now possible to obtain accurate images regarding dimension, composition and structure of the plaque. At the same time, because the plaques can be removed by surgery, it becomes necessary to establish which are potentially harmful.

Therefore, it is desirable to establish a correlation between the instrumental signals, especially the Doppler signals, and the real risk entity. To establish this, the plaque must be analysed by Doppler techniques, removed, and then studied using techniques that can provide information concerning their composition, structure and stability, with special reference to the inorganic salts. Finally, any correlation between this information and the data of the Doppler analysis must be assessed.

Instrumental techniques such as thermal analysis, atomic absorption spectroscopy, ICPS analysis and infrared spectroscopy were used to carry out this study.

EXPERIMENTAL

Apparatus

The TG, DTG and DSC curves were obtained by using a Perkin–Elmer 7 Series apparatus. The heating rate used was $10\,^{\circ}C\ min^{-1}$. The furnace atmosphere consisted of either dry nitrogen or air at flow rates of 50–100 ml min^{-1}. The IR spectra were obtained by using a Perkin–Elmer 1760 FT-IR research system. The analysis of plaque cation contents was carried out by atomic absorption spectroscopy using a Perkin–Elmer 5000 apparatus.

RESULTS

Plaques from carotid vessels were analysed following surgery. The best means of transport and preservation were first studied. Both fresh plaque samples and plaque that had been preserved in different ways were dissected

and analysed. The best preservation technique was quenching in liquid nitrogen immediately after collection, followed by storage at $-40\,^{\circ}C$. Samples treated in this way gave instrumental signals identical with those of the same fresh samples. 58 plaques previously analysed by "in vivo" Doppler techniques were studied by thermal analysis.

The TG curves, Figs. 1–3, show that the plaques decompose in three main processes: the first corresponding to water loss, followed by two processes corresponding to the breakdown of the organic matrix, yielding a residue of inorganic salts.

It is interesting to note that, as shown either by the DTG or DSC curves, water is not isoenergetically bound to the organic matrix but, as shown by a convolution of the superimposed processes, different types of water with different energy bonds are present. The TG curves show that the water content and residue can vary drastically, although the trend of the decomposition behaviour does not change with different plaques. In particular, the content of mineral salts can be higher than 50%.

Sometimes the same plaque can show dramatic differences in the inorganic salt content moving from the border to the centre of the plaque, as shown in Figs. 4 and 5.

In order to obtain information about the composition of the inorganic salts in the plaques, the residues from the TG analyses were recovered and

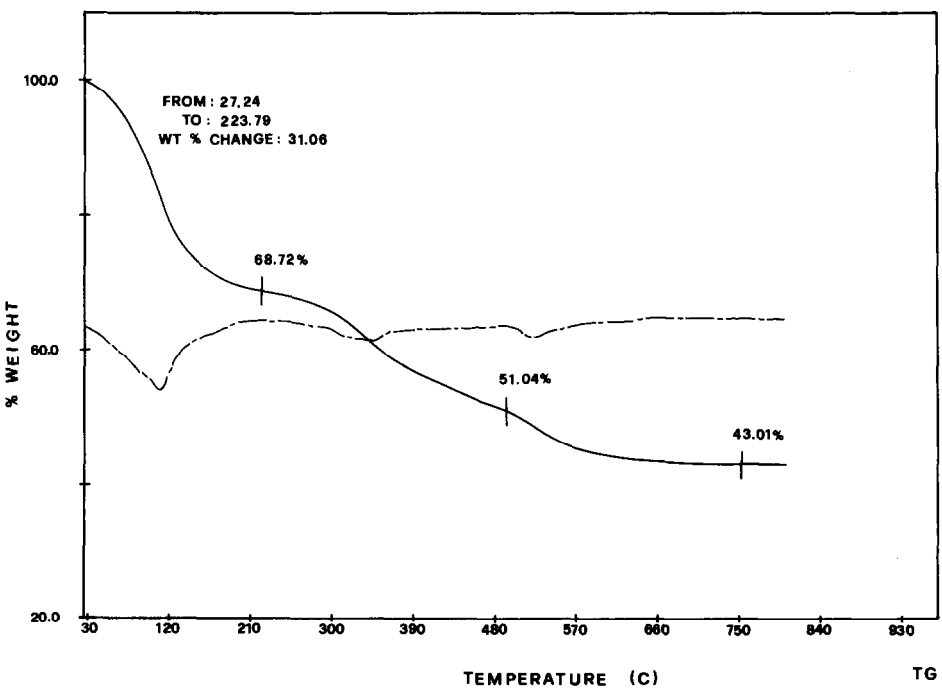

Fig. 1. TG and DTG curves of hard plaque: air atmosphere; heating rate $10\,^{\circ}C\ min^{-1}$.

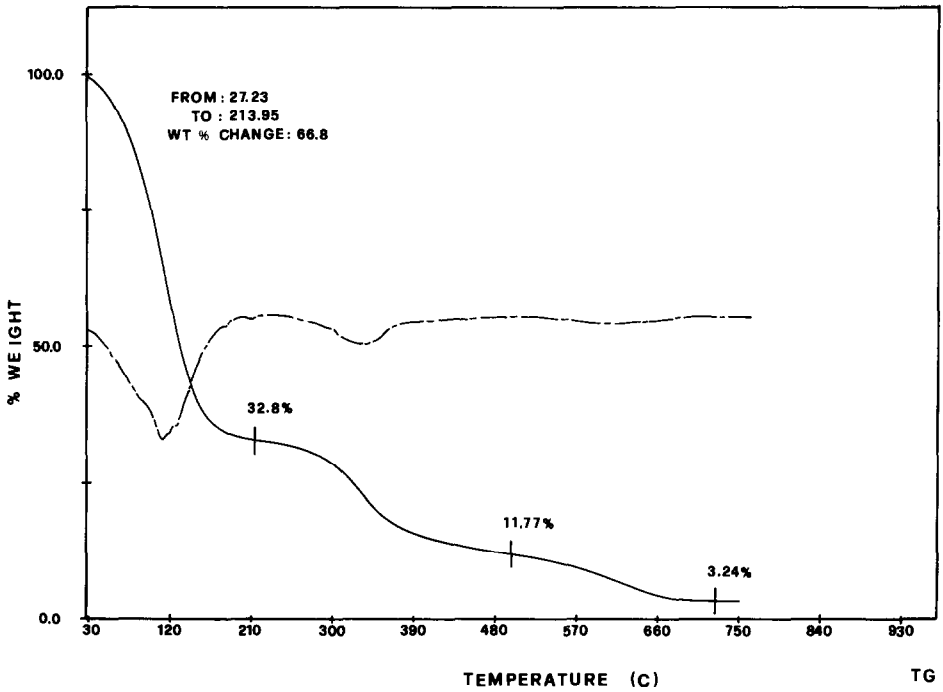

Fig. 2. TG and DTG curves of medium plaque: air atmosphere; heating rate 10 °C min^{-1}.

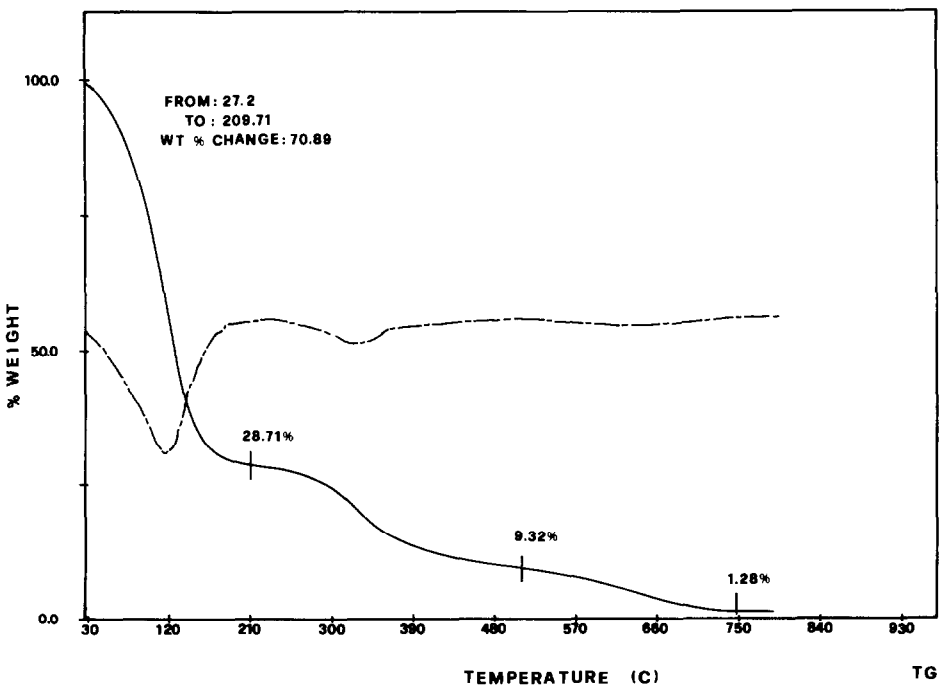

Fig. 3. TG and DTG curves of soft plaque: air atmosphere; heating rate 10 °C min^{-1}.

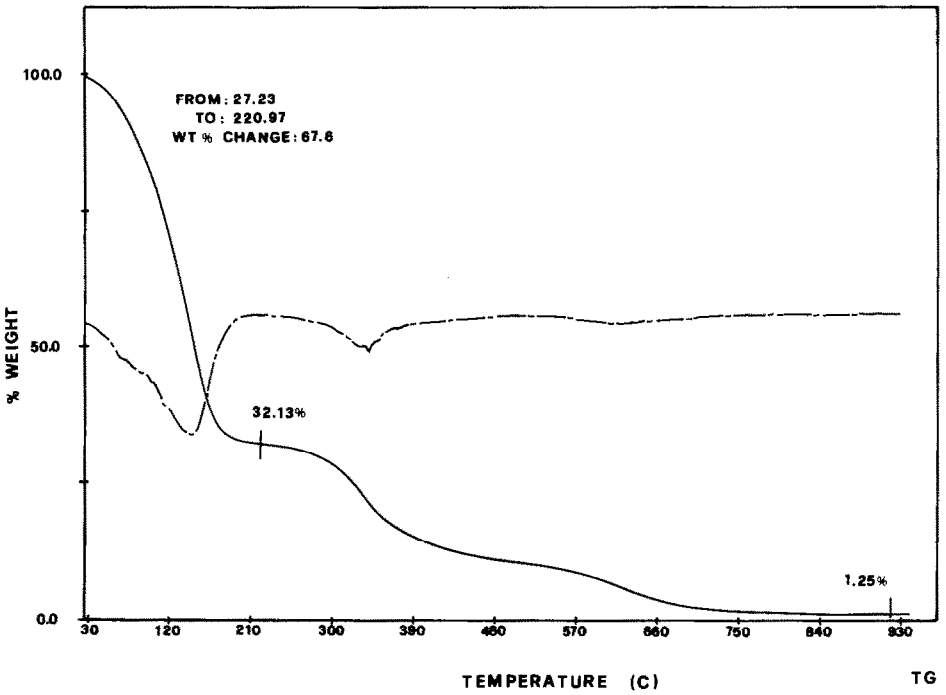

Fig. 4. TG and DTG curves of border of plaque: air atmosphere; heating rate 10 °C min^{-1}.

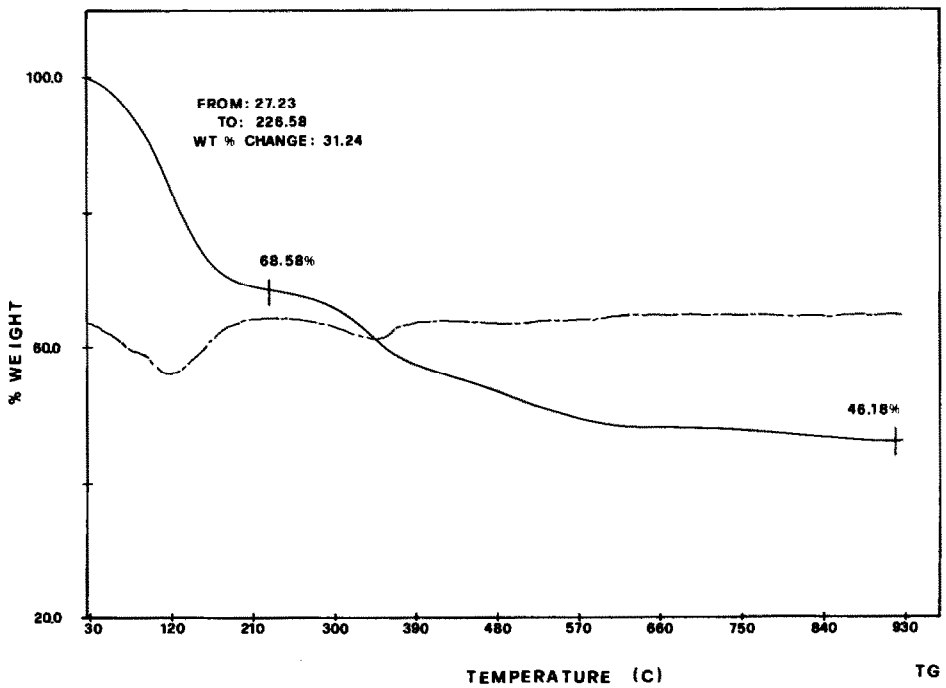

Fig. 5. TG and DTG curves of centre of the same plaque as Fig. 4: air atmosphere; heating rate 10 °C min^{-1}.

TABLE 1

Atheromasic plaques, analysis by A.A.S. of the TG residues (mg g^{-1} of plaque)

	Res. (%)	Mg	Ca	Zn	Cu	Ca/Mg	Ca/Zn	Ca/Cu	Mg/Zn	Mg/Cu	Cu/Zn
1	1.24	0.28	4.25	0.06	ldl [a]	15	71	ldl [a]	5	ldl [a]	ldl [a]
2	43.01	2.15	165.90	0.15	ldl	77	1106	ldl	14	ldl	ldl
3	13.19	0.67	50.13	0.03	ldl	75	1670	ldl	22	ldl	ldl
4	2.42	0.36	8.8	ldl	ldl	24	ldl	ldl	ldl	ldl	ldl
5	1.29	0.29	4.6	0.02	ldl	16	230	ldl	14.5	ldl	ldl
6	2.21	0.36	8.1	0.03	1.06	22	270	7.6	12	0.34	35
7	44.14	2.0	169.1	0.1	0.06	85	1691	2818	20	33	0.6
8	3.58	0.29	13.4	0.03	ldl	46	447	ldl	10	ldl	ldl
9	1.14	0.34	3.9	0.03	ldl	11	130	ldl	11	ldl	ldl
10	2.57	0.17	9.7	0.01	0.13	57	970	75	17	1.3	13
11	42.51	1.90	162.9	0.12	0.06	86	1357	2715	16	32	0.5
12	3.27	0.51	11.9	0.03	ldl	23	396	ldl	17	ldl	ldl
13	3.15	0.27	11.7	0.02	ldl	43	585	ldl	14	ldl	ldl
14	2.53	0.45	9.2	0.03	0.11	20	306	84	15	4	4
15	37.69	2.18	142.1	0.15	0.09	65	947	1578	14	24	0.6
16	55.49	2.10	206.3	0.20	0.06	98	1031	3438	10	35	0.3

[a] Below detection limit.

TABLE 2

Calcium/magnesium ratio in the plaques

Hard			Medium			Soft		
Sample	Ca/Mg	Res. (%)	Sample	Ca/Mg	Res. (%)	Sample	Ca/Mg	Res. (%)
2	77	43.01	8	46	3.58	1	15	1.24
3	75	13.19	10	57	2.57	4	24	2.42
7	85	44.14	12	23	3.27	5	16	1.29
11	86	42.51	13	43	3.15	6	22	2.21
15	65	37.69	22	47	3.85	9	11	1.14
16	98	55.49	31	45	3.68	14	20	2.53
21	82	48.73	35	39	3.41	23	15	1.25
25	79	17.08	48	30	2.51	24	15	1.10
17	90	37.72	49	46	3.72	27	18	1.70
38	80	45.77	53	48	3.15	28	16	1.03
20	77	41.93				29	18	1.20
40	76	25.72				30	21	0.79
42	78	11.33				32	24	1.58
43	82	12.09				33	16	0.95
44	90	54.53				34	24	1.38
46	81	31.10				36	23	1.24
47	92	63.19				32	16	0.99
50	74	46.21				39	15	0.58
52	73	39.29				41	17	1.61
54	84	58.44				51	16	1.24
57	80	40.76				55	15	1.51
58	78	33.54				56	24	1.01
Average	81		Average	42		Average	18	

analysed by ICPS and atomic absorption spectroscopy. Using these techniques, it was possible to indicate, as shown by the example given in Table 1, that Ca^{2+} and Mg^{2+} are the predominant ions and that sometimes Cu^{2+} and Zn^{2+} are also present in notable amounts.

IR spectroscopy of the same residues, thus avoiding possible interferences from the organic matrix, revealed bands characteristic of phosphates [4,5]. The quantitative analysis showed that these anions are the main counteranions for the cations found in the residues, at levels of about 92%. The X-ray analysis of many calcific masses has shown that these systems tend to form hydroxyapatite.

DISCUSSION

As shown by the thermoanalytical curves, the water molecules present in the examined samples were not isoenergetically bound to the matrix, but different kinds of water with different bond energies were present. This

phenomenon agrees perfectly with Watterson's hypothesis [6–8] that water in biological systems is bound to the other components of the matrix through hydrogen bonds, van der Waal forces, London forces, etc., and takes part in mutual interactions on a macromolecular scale. These mutual interactions evolve toward the formation of clusters, that have a relatively long lifetime and that stabilise strongly the water molecules in the system. The different clusters show different interaction energies with their surroundings and this determines, at the thermochemical level, the appearance of different signals at different temperatures corresponding to the different types of water present in the system. A statistical analysis demonstrates that as the residue and then the inorganic salt content in the plaques increases, the water content decreases, particularly the water with a higher bond energy, indicating that the water clusters at higher bond energy may be being substituted by clusters of inorganic salts.

Thermal analysis demonstrates that the plaques can be divided into three groups with respect to their residue content as obtained by TG analysis: hard plaques (Fig. 1); medium plaques (Fig. 2); and soft plaques (Fig. 3).

Concerning the residues, the ratio of calcium to magnesium concentration in the different plaques showed that with increasing residue content and, consequently, increasing hardness of the plaque, the calcium/magnesium ratio also increases, ranging from average values of 18 for the soft plaques, of 42 for the medium plaques and of 81 for the hard plaques, see Table 2. The calcification of plaques is quite a common event, although the reasons for this process are not fully clear. It has been suggested that this phenomenon is controlled by those same mechanisms which are implicated in dystrophic calcification of other body tissues.

Large calcific areas are commonly associated with plaques showing extensive necrosis, whereas microcalcifications are sometimes present in smaller plaques.

Calcium salts are mainly present as small masses which grow to ossified areas with time.

Local factors could promote the precipitation of calcium and magnesium ions, and the presence of magnesium could stabilise the amorphous phase of calcium, while on the other hand the disappearance of the magnesium could lead to a crystallisation of the calcium bound to the phosphates, leading to a chemical composition very similar to that of bone hydroxyapatite.

From the preliminary data, according to the epidemiological investigations concerning the incidence of ictus, these seems to be a correlation with the type of plaque present in the patients: the plaques at higher risk are the soft–medium type. The highest risk is associated with the medium plaques in which calcification is beginning to manifest itself, originating in microformed systems that are not as well aggregated as hydroxyapatitic nodules and that constitute disaggregation points of the system, suggesting a lower dynamic resistance.

ACKNOWLEDGEMENTS

The support of Regione Marche and MPI (40%) is gratefully acknowledged. The authors thank Mr Stefano Marazzi for technical assistance.

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