In vivo production of prostacyclin and thromboxane in patients with acute myocardial infarction

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SUMMARY The in vivo production of prostacyclin and thromboxane was monitored by measuring their major urinary metabolites 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ in ten patients with acute myocardial infarction, five on standard treatment and five receiving prostacyclin infusion. During acute myocardial infarction excretion of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, measured by a gas chromatography-mass spectrometry method with deuterated internal standards, was significantly increased. This indicates that thromboxane and prostacyclin synthesis are increased during the development of acute myocardial infarction. The excretion data for 2,3-dinor-thromboxane B_2 showed that after administration of aspirin there was less pronounced and more variable inhibition than expected. Prostacyclin infusion did not markedly affect the excretion of the thromboxane metabolite.

There has been considerable interest in the possible role of platelets in the pathogenesis of acute myocardial infarction.[1] The discovery of thromboxane A_2 and its vasoconstricting and platelet aggregating effects has focused attention on the possible involvement of this platelet-derived compound in cardiovascular disease.[2] Similarly the discovery of prostacyclin and its vasodilatory and platelet antiaggregatory effects triggered off intense research on the possible role of this compound in protective mechanisms against cardiovascular disease.[3]

Studies on the possible involvement of those compounds in human cardiovascular disease have long been hampered by the lack of suitable methods for monitoring the in vivo production of thromboxane A_2 and prostacyclin. Thromboxane A_2 and prostacyclin are chemically degraded in vivo to thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ respectively. It is highly questionable, however, whether measurement of thromboxane B_2 in blood reflects thromboxane A_2 synthesis in vivo.[4] Increasing evidence also suggests that concentrations of 6-keto-prostaglandin $F_{1\alpha}$ found in samples of peripheral blood do not reflect the formation of prostacyclin in vivo.[5-8]

The in vivo metabolism of thromboxane B_2 and prostacyclin has been studied in man and the main urinary metabolites have been identified as 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ respectively.[9 10] Methods for analysis of these compounds have been developed based on deuterated internal standards/carriers and gas chromatography-mass spectrometry.[11-14] Excretion of those metabolites quickly (0·5–1·5 hours) and adequately reflects changes in the production of thromboxane A_2 and prostacyclin in vivo.[7 14-16] The intra-individual variation in the excretion of these metabolites is small and independent of urine production.[16] The excretion of the prostacyclin metabolite is, however, considerably increased by physical exercise.[14]

We report on the formation of thromboxane A_2 and prostacyclin in vivo during and after the development of myocardial infarction.[17]

Patients and methods

All patients were admitted to the coronary care unit less than seven hours after the onset of chest pain. A diagnosis of myocardial infarction was confirmed by creatine kinase MB estimations every four hours. Five patients were given placebo infusion and five had prostacyclin infusion (4–5 ng/kg/min) for 96 hours as well as conventional treatment.[18] Urine was

collected continuously before and every eight hours during the infusion period and was analysed for 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$. This design was selected in order to study not only the magnitude but also the dynamics of the synthesis of thromboxane and prostacyclin. A 12 hour urine collection was obtained from most individuals seven to nine months after the acute myocardial infarction.

The methods that we used to measure 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ in urine have been described in detail elsewhere.[13 14]

Results

The Table shows the results from the initial chest x ray investigation as well as the medication given to each individual as a result of the clinical decision reached during the first three days after admission. All patients were given 0·5 g of aspirin and 225 mg dipyridamole 72 hours after the start of the infusion as a part of the study protocol. Figure 1 shows the urinary excretion of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ from the five patients (cases A–E) on placebo infusion and Fig. 2 their excretion from patients (F–J) on prostacyclin. The excretion rate of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ in normal healthy individuals is 130–535 pg/mg creatinine and 38–508 pg/mg creatinine respectively.[13 14] Descrip-

tions of the individual cases are given below. Apart from aspirin none of the conventional drugs (such as morphine and frusemide) that were given affected the excretion of the thromboxane and prostacyclin metabolites.

Patient A—The initial excretion of 2,3-dinor-thromboxane B_2 was clearly higher than that seen in normal males. The patient received 500 mg of aspirin because of pain 9, 15, and 22 hours after the start of infusion. This reduced the excretion of 2,3-dinor-thromboxane B_2 by about half to 378 pg/mg creatinine. Another dose of 500 mg aspirin at 69 h caused a further reduction from 205 to 124 pg/mg creatinine. Urinary excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ was initially within the limits for normal healthy males, but there was a continuous increase in concentrations up to 3110 pg/mg creatinine from seven to 15 hours after the maximum creatine kinase MB concentration.

Patient B—Before and during the first 2·5 days of infusion urinary excretion of 2,3-dinor-thromboxane B_2 was two to four times higher than the highest excretion seen in normal healthy females. Sixty one hours after the start of the infusion she was given 500 mg of aspirin, and 4–12 hours afterwards excretion of 2,3-dinor-thromboxane B_2 increased to 5646 pg/mg creatinine. This patient initially had normal urinary excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ that later increased to a peak of 3605 pg/mg creatinine. Nine months later excretion of 2,3-dinor-thromboxane B_2 and 2,3-dinor-6-keto-

Table   *Medication in study group*

| Case No	Initial chest x ray findings	Day 1	Day 2	Day 3
A	Congestive heart failure	Morphine 28 mg iv, practolol 2 mg iv, frusemide 120 mg iv, aspirin 1·5 g oral	Frusemide 80 mg oral	Frusemide 80 mg oral
B	Normal	Morphine 17·5 mg iv, frusemide 120 mg iv	Morphine 5 mg iv	Metoprolol 150 mg oral
C	Normal	0	Metoprolol 150 mg oral	Metoprolol 150 mg oral
D	Normal	Pethidine 25 mg iv, frusemide 20 mg iv	Metoprolol 150 mg oral	Metoprolol 150 mg oral
E	Enlarged	Morphine 10 mg iv	Metoprolol 100 mg oral	Metoprolol 100 mg oral
F	Enlarged	Morphine 20 mg iv, frusemide 20 mg iv, metoprolol 200 mg oral	Metoprolol 200 mg oral	Metoprolol 200 mg oral
G	Congestive heart failure	Morphine 18 mg iv, frusemide 80 mg iv	Morphine 20 mg iv, aspirin 8 g oral, hydrocortisone 100 mg iv, metoprolol 150 mg oral	Aspirin 8 g oral, metoprolol 150 mg oral
H	Congestive heart failure	Morphine 38 mg iv, frusemide 80 mg iv	Frusemide 40 mg oral, atenolol 100 mg oral, aspirin 1·5 g oral	Frusemide 40 mg oral, atenolol 100 mg oral, aspirin 1·5 g oral
I	Congestive heart failure	Pethidine 25 mg iv, ketobemidone 10 mg iv, frusemide 100 mg iv	Aspirin 0·5 g + 1·0 g oral	Frusemide 40 mg iv, aspirin 2·0 mg oral
J	Congestive heart failure	Morphine 5 mg iv, frusemide 20 mg iv	0	Frusemide 80 mg oral
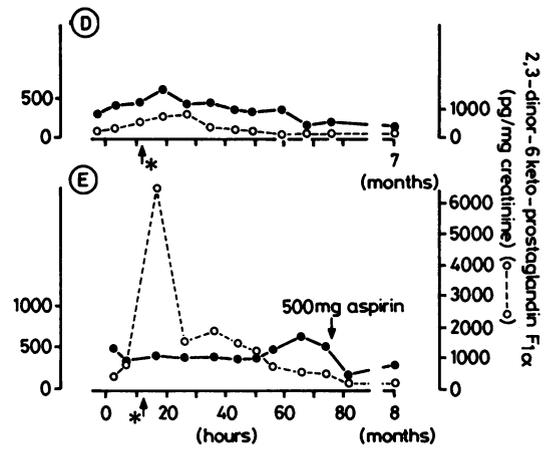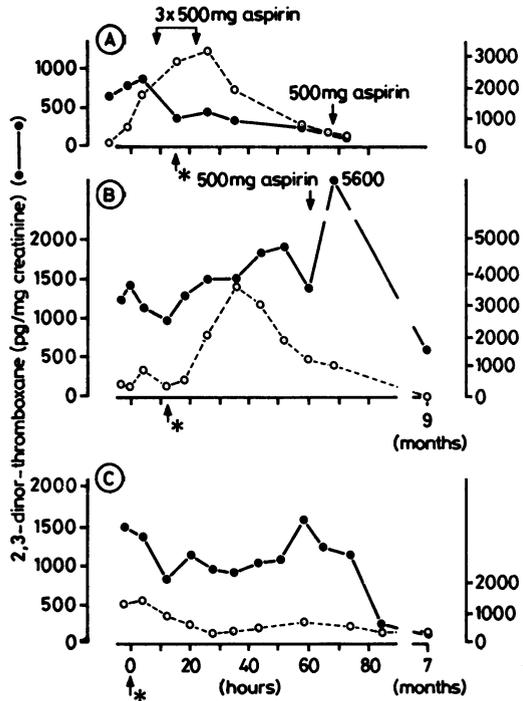
Fig. 1   *Urinary excretion of 2,3-dinor-thromboxane B₂ and 2,3-dinor-6-keto-prostaglandin F₁α in patients on a placebo infusion. 0 h, indicates start of infusion. Each data point along the time axis is plotted for the middle of each period of urine collection. Arrow with asterisk indicates the time at which creatine kinase MB was highest. In adults normal excretion of 2,3-dinor-thromboxane B₂ is 130–535 pg/mg creatinine and of 2,3-dinor-6-keto-prostaglandin F₁α it is 38–508 pg/mg creatinine (n = 20 both groups).*
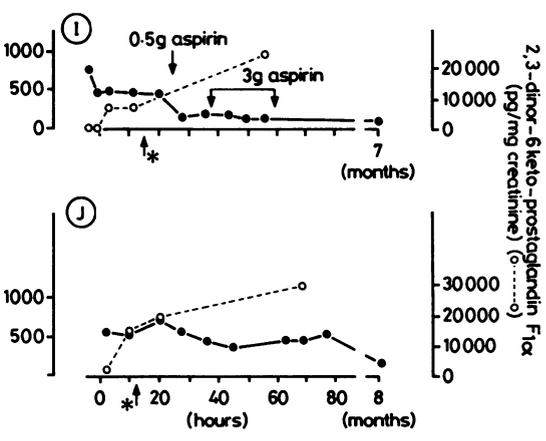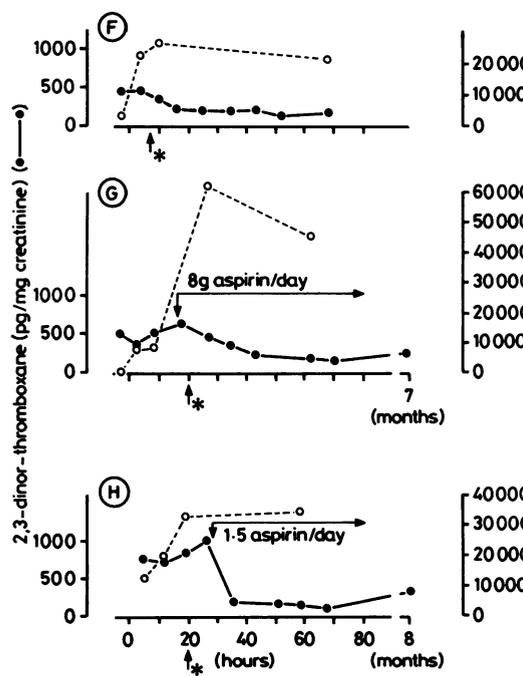
Fig. 2   *Urinary excretion of 2,3-dinor-thromboxane B₂ and 2,3-dinor-6-keto-prostaglandin F₁α in patients on a prostacyclin infusion. 0 h, see legend to Fig. 1 for explanation.*

prostaglandin $F_{1\alpha}$ was 636 and 86 pg/mg creatinine respectively.

Patient C—Excretion of 2,3-dinor-thromboxane B_2 in all urine samples but the last one was higher than that seen in normal healthy males. The peak value, 54–62 hours after the start of infusion, coincided with three episodes of angina pectoris. Seven months later excretion of 2,3-dinor-thromboxane B_2 was 143 pg/mg creatinine. In this patient excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ reached a peak (1405 pg/mg creatinine) shortly after the maximal creatine kinase MB value. Seven months later the excretion was 358 pg/mg creatinine.

Patient D—The urinary excretion of 2,3-dinor-thromboxane B_2 in this patient was initially 302 pg/mg creatinine and increased to twice this value around 10 h after the maximum creatine kinase MB value. Excretion seven months later was 192 pg/mg creatinine. Urinary excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ was initially within normal limits for healthy males. It then increased to a maximum of 755 pg/mg creatinine. Seven months later excretion was 119 pg/mg creatinine.

Patient E—Urinary excretion of 2,3-dinor-thromboxane B_2 was within the normal range until about 50 hours after creatine kinase MB concentration reached a maximum, then it increased to a peak of 650 pg/mg creatinine. A dose of aspirin (500 mg) reduced the urinary excretion rate of 2,3-dinor-thromboxane B_2 by about 70%. Eight months later urinary excretion was 318 pg/mg creatinine. The initial urinary excretion rate of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ was within the normal range but it then increased abruptly to a maximum of 6348 pg/mg creatinine. This abrupt increase was not associated with any obvious clinical symptom. Eight months later urinary excretion was 240 pg/mg creatinine.

PROSTACYCLIN (EPOPROSTENOL) INFUSION
After the start of prostacyclin infusion the excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ increased to around 30 000 pg/mg creatinine.

Patient F—In the first urine samples the urinary excretion of 2,3-dinor-thromboxane B_2 was 459 and 472 pg/mg creatinine and thereafter the excretion fell to around 200 pg/mg creatinine. Urinary excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ was increased in the first urine sample, probably because the infusion was started 0·5 hour before the collection of the first urine sample was completed. As expected excretion increased even more during the infusion.

Patient G—Urinary excretion of 2,3-dinor-thromboxane B_2 was initially somewhat raised (peak

value 630 pg/mg creatinine). The development of a severe pericarditis (at about 20 hours) required treatment with 8 g of aspirin daily combined with injection of 100 mg hydrocortisone. Despite this large dose of aspirin urinary excretion of 2,3-dinor-thromboxane B_2 only decreased slowly to about 30% of the maximum level. Seven months later the excretion of 2,3-dinor-thromboxane B_2 was 249 pg/mg creatinine.

Patient H—During the first day of prostacyclin infusion the urinary excretion rate of 2,3-dinor-thromboxane B_2 was clearly higher than the excretion rate in normal healthy males. Twenty eight hours after the start of the infusion the patient was started on 1·5 g aspirin daily for pericarditis. An abrupt reduction in excretion (by about 80%) followed. Eight months later excretion of the thromboxane metabolite was 320 pg/mg creatinine.

Patient I—Urinary excretion of 2,3-dinor-thromboxane B_2 in the initial sample was clearly higher (755 pg/mg creatinine) than that in normal healthy males. The patient received 500 mg aspirin at about 25 hours, and this reduced urinary excretion of 2,3-dinor-thromboxane B_2 by 50–60%. Later the patient was given more aspirin (3 g in total) without any further reduction of the excretion of 2,3-dinor-thromboxane B_2. The excretion of 2,3-dinor-thromboxane B_2 seven months later was 115 pg/mg creatinine.

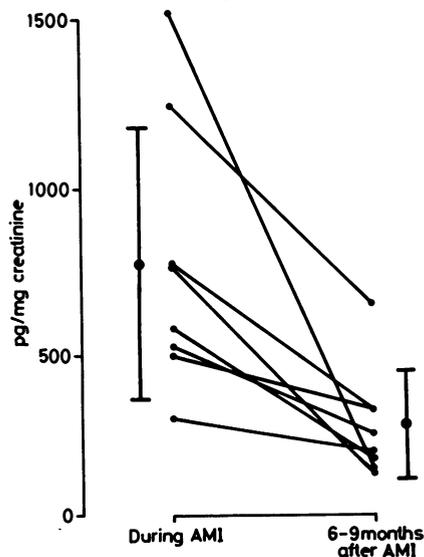*Patient J*—The urinary excretion of 2,3-dinor-thromboxane B_2 was slightly higher than

Fig. 3 *Urinary excretion of 2,3-dinor-thromboxane B_2 in the first sample collected after acute myocardial infarction (AMI) and in a sample collected 6–9 months later in eight individuals. Individual values are means (SD).*

normal for most of the study. Eight months later the excretion of 2,3-dinor-thromboxane B_2 was 173 pg/mg creatinine.

STATISTICAL EVALUATION

We compared the initial and follow up values on the excretion of 2,3-dinor-thromboxane B_2 in the eight cases in which we were able to collect follow up samples (Fig. 3). The mean initial excretion was 771 (410) pg/mg creatinine (mean (SD)) and the follow up excretion was 268 (167) pg/mg creatinine (mean (SD)). Student's t test for paired samples showed a highly significant difference ($p < 0.01$) between the initial and follow up excretion.

Discussion

We found a prolonged increase in the in vivo synthesis of thromboxane and prostacyclin during the acute phase of myocardial infarction. This suggests that these substances may be involved in the pathophysiology of acute myocardial infarction.

In all patients except case F excretion of 2,3-dinor-thromboxane B_2 was higher than normal during at least part of the study period. Since there is little variation (SD 10–23%) in the excretion of 2,3-dinor-thromboxane B_2 in a normal individual over weeks and months,[16] we believe that the finding that maximum increases seen during the study were 2–11 times higher than those found 7–9 months later is more important than the general rise in excretion. There was also a highly significant difference between the excretion in the very first urine sample, which was collected before any treatment was started, and in that obtained 7–9 months later. Moreover the excretion in one and the same individual during the first 80 hours was very variable; the ratio between the highest and lowest values ranged from about 1·5:1 to 3·5:1 (values for case A and after aspirin are not included). Thus both the level of and the variability of the excretion of 2,3-dinor-thromboxane B_2 strongly suggest the involvement of thromboxane in the pathogenesis of acute myocardial infarction.

Excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ in four of the cases without prostacyclin infusion was initially within the normal range. Thereafter the excretion increased, reaching a peak 2–20 hours after the maximum creatine kinase MB value. Excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ varies in healthy individuals (intra-individual variation 16–31% SD[16]) and increases considerably during physical exercise.[14] Despite the fact that our patients remained in bed, the maximum concentrations seen in cases A, B, C, and E were far above (or in case D, close to), concentrations found in normal individuals during strenuous exercise. In all cases (A–J) excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ seven to nine months later was within the range seen in normal individuals. Thus prostacyclin synthesis in vivo is increased during an acute myocardial infarction. Synthesis is greatest soon after maximal disintegration of myocardial tissue, as indicated by the appearance of creatine kinase MB in the circulation.

Excretion of 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ before infusion of prostacyclin was within normal limits and increased to about 30 000 pg/mg creatinine during infusion. The infusion had no obvious immediate effect on the excretion of the thromboxane metabolite. These data seem to contradict the idea that exogenous prostacyclin will reduce in vivo thromboxane synthesis.

Three individuals in each group were given aspirin during the study. Only three of them, E, H, and I, showed an abrupt reduction in 2,3-dinor-thromboxane B_2 excretion (by 65%, 82%, and 65% respectively). These values are similar to or only slightly higher than those seen in normal individuals after 500 mg of aspirin. The effect of aspirin on the excretion of 2,3-dinor-thromboxane B_2 in cases A, B, and G, however, is distinctly different from that seen in normal individuals.[13 16] In case A a dose of 3×500 mg aspirin caused a 55% decrease in urinary 2,3-dinor-thromboxane B_2 from 880 to about 378 pg/mg creatinine. A further 500 mg aspirin given at 69 h caused another reduction from 205 to 124 pg/mg creatinine. Patient B was given 500 mg aspirin at 61 hours when symptoms of what later turned out to be a post-myocardial infarction syndrome first appeared. The urine sample collected after this showed an enormous increase in the excretion of 2,3-dinor-thromboxane B_2. Patient G developed symptoms of pericarditis around 20 hours after the start of infusion and daily doses of 8 g aspirin were given. Despite this large dose excretion of 2,3-dinor-thromboxane B_2 declined only gradually to around 150 pg/mg creatinine. Aspirin has less effect on thromboxane synthesis during acute myocardial infarction (that is when thromboxane synthesis seems to be raised and labile) than it does in healthy individuals.

Our data strongly indicate that synthesis of thromboxane is increased and variable for several days after acute myocardial infarction. This probably reflects a generalised increase in platelet aggregability but we cannot rule out the possibility of an increase in thromboxane synthesis when the coronary vessel becomes occluded. We can only speculate on the reasons for a very high synthesis of prostacyclin, which peaked after maximum creatine kinase MB release, but this finding could indicate

that most of the increase in endogenous prostacyclin synthesis is the result of tissue necrosis or even repair. The excretion data collected after administration of prostacyclin and aspirin raise several new questions about the prevention of acute myocardial infarction and reduction of infarct size.[19] The actions of aspirin and or other thromboxane synthetase inhibitors on the synthesis of thromboxane A_2 under pathological conditions clearly cannot be forecast from results obtained under normal conditions. Studies are continuing.

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