

Platelet sensitivity to prostacyclin in normal subjects, and in patients with benign and malignant tumours of the breast

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Summary Platelet sensitivity to prostacyclin (PGI₂) was determined in normal male and female subjects, and in patients with benign and malignant tumours of the breast.

The IC₅₀ overall mean values for PGI₂ on ADP-induced platelet aggregation were similar for normal men and women, being $0.97 \pm 0.05 \text{ ng ml}^{-1}$ and $0.83 \pm 0.07 \text{ ng ml}^{-1}$ respectively. However, there were significant differences in the IC₅₀ values for women in the 1st ($0.81 \pm 0.06 \text{ ng ml}^{-1}$) vs. 2nd ($1.37 \pm 0.13 \text{ ng ml}^{-1}$) phase of the menstrual cycle; post-menopausal women gave similar values to normal males and to pre-menopausal women in the 1st phase of the cycle.

No significant differences were found between normal subjects and patients with benign or malignant tumours of the breast when account was taken of the status of the patient in relation to the phase of the menstrual cycle and the menopause.

The importance of the hormonal status in evaluating changes in platelet sensitivity in patients with breast cancer is strongly emphasised.

Prostacyclin (PGI₂) may play an important role in the control of vascular and platelet homeostasis (Bunting *et al.*, 1983). Based upon the assumption that platelet-tumour cell and/or platelet-tumour cell-vessel wall interactions can influence the metastasis of tumour cells, Honn and coworkers (Honn, 1982; Honn *et al.*, 1983) suggested that the presence of malignant tumours could disrupt the intravascular balance between PGI₂ and thromboxane A₂ (TXA₂), the main endogenous antagonist of PGI₂ (Bunting *et al.*, 1983), in favour of platelet aggregation, thus favouring the likelihood of metastasis.

Another factor that may contribute to changes in platelet aggregation in patients with tumours is the sensitivity of the platelets to PGI₂. This aspect may be investigated *in vitro* using synthetic PGI₂, and probably is an essential part of the biological activity of PGI₂ through its binding to platelet membranes, and in the regulation of haemostatic balance (Schillinger & Prior, 1980).

Since studies on humans have demonstrated that patients with colonic cancer have a decreased platelet sensitivity to PGI₂ (Gisinger *et al.*, 1982) and malignant tumours of the breast produce substantial amounts of PGI₂ (Bennett *et al.*, 1983) we

decided to investigate the platelet sensitivity to PGI₂ in patients with benign and malignant tumours of the breast.

Subjects and methods

Subjects

Four groups of subjects have been studied:

- 1) 26 healthy normal female subjects;
- 2) 14 patients with benign tumours of the breast;
- 3) 30 patients with malignant tumours of the breast;
- 4) 16 men as healthy controls.

The range and mean values \pm s.d. of ages of all subjects and the number of women in pre- and post-menopause are given in Table I. None of the subjects was on oral contraceptives and 7 healthy women in pre-menopause were studied both in the first and second phase of the same cycle. In 4 normal subjects the estimations were repeated during different menstrual cycles. Eight men, 1 woman with a malignant tumour and 1 with a benign tumour of the breast smoked more than 10 cigarettes a day; none of the subjects had taken drugs known to affect prostaglandin metabolism in the two weeks before the blood sampling, or had diabetes mellitus or coronary heart diseases. In the group with malignant tumours 3 patients had hypertension, 3 were obese.

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Table I Clinical data on the subjects studied. For details see the text; mean values are given \pm s.d.

Groups of subjects	Age (years)		Total	Pre-menopause		Post-menopause (n)
	Pre-menopause	Post-menopause		1st phase of the cycle (n)	2nd phase of the cycle (n)	
1. Healthy women (n=26)	28 \pm 4 (24–37)	54 \pm 4 (48–61)	41 \pm 14 (24–61)	12	8	13
2. Women with benign breast tumours (n=14)	39 \pm 6 (31–49)	—	39 \pm 6 (31–49)	6	8	—
3. Women with malignant breast tumours (n=30)	45 \pm 7 (33–35)	61 \pm 10 (45–77)	53 \pm 12 (33–77)	6	8	16
4. Healthy men (n=16)	—	—	31 \pm 8 (25–54)	—	—	—

Histopathological data on benign and malignant tumours

The benign tumours of the breast were all fibroadenomas. The malignant tumours included: 20 infiltrating duct carcinomas, 4 infiltrating lobular carcinomas, 1 colloid papillary infiltrating carcinoma, 1 colloid infiltrating carcinoma, 2 in situ duct carcinomas and 2 in situ lobular carcinomas. Eleven tumours had a maximum diameter greater than 2 cm and lymph node metastases were present in 16 patients.

Blood sampling

Blood samples were always taken between 8 and 9 a.m.; the subjects had fasted overnight prior to the sampling.

The blood was drawn from the antecubital vein with a polypropylene syringe via a Butterfly needle No. 21 (Abbott Ireland Ltd., Sligo, Rep. of Ireland), placed in a plastic tube containing one tenth of its volume of 3.3% (w/v) trisodium citrate and quickly mixed.

Preparation of platelet-rich and platelet-poor plasma

Platelet-rich plasma (PRP) was obtained by centrifuging blood at 150 g for 12 min. Platelet-poor plasma (PPP) was prepared by centrifugation of the remaining blood at 2000 g for 15 min. The platelet count of PRP was adjusted to 250×10^3 cells μl^{-1} by dilution with homologous PPP. The PRP and PPP were kept at room temperature ($\sim 22^\circ\text{C}$).

Platelet sensitivity to PGI₂

The platelet sensitivity to PGI₂ in the plasma was

determined using a modification of the method of Sinzinger *et al.* (1981).

Platelet aggregation was studied using 250 μl of PRP in a double-channel Elvi aggregometer at 37°C with constant stirring. Following a preincubation period of 15 sec, 20 μl of different concentrations of standard PGI₂ in Tris buffer (50 μM , pH 9.5) were added to the PRP 1 min before the addition of ADP (final concentration 1 μM).

The platelet sensitivity to prostacyclin was expressed as the dose of the synthetic PGI₂ (in ng ml^{-1} PRP) necessary to suppress by half the aggregation induced by ADP (IC₅₀).

Chemicals

ADP was obtained from Semmelweis s.r.l.-Mascia Brunelli (Milano, Italy). PGI₂ standards were kindly provided by Dr J. Pike (Upjohn Company, Kalamazoo, Michigan, U.S.A.) and by Dr B.J.R. Whittle (Wellcome Foundation, Beckenham, Kent, U.K.).

Statistics

Statistical evaluations were made by Student's *t*-test. Differences and correlations were considered significant when $P < 0.05$.

Results

The mean values \pm s.e. of the IC₅₀ for prostacyclin on the ADP-induced platelet aggregation assay are shown in Table II for the 4 groups of subjects studied: where appropriate, the groups are subdivided so as to separate values found during the

Table II Mean values \pm s.e. for the IC_{50} in $ng\ ml^{-1}$ that corresponds to the inhibitory effect of PGI_2 on ADP-induced platelet aggregation. *Group 1*: normal female control subjects; *Group 2*: patients with benign breast tumours; *Group 3*: patients with malignant breast tumours; *Group 4*: normal male subjects. Where appropriate, the values have been sub-divided to indicate where samples were obtained during the 1st or 2nd phases of the menstrual cycle, and after the menopause. For further details see the text.

Groups of subjects	$PGI_2\ IC_{50}\ ng\ ml^{-1}$				
	All values (a)	1st phase of cycle (b)	2nd phase of cycle (c)	1st & 2nd phases of cycle (d)	Post-menopause (e)
1. Healthy women	0.97 ± 0.05 (n=39)	0.81 ± 0.06 (n=16)	1.37 ± 0.13 (n=10)	1.02 ± 0.08 (n=26)	0.86 ± 0.04 (n=13)
2. Women with benign breast tumours	1.06 ± 0.08 (n=14)	0.86 ± 0.10 (n=6)	1.21 ± 0.10 (n=8)	1.06 ± 0.08 (n=14)	—
3. Women with malignant breast tumours	0.99 ± 0.06 (n=30)	0.83 ± 0.13 (n=6)	1.18 ± 0.20 (n=8)	1.03 ± 0.12 (n=14)	0.96 ± 0.06 (n=16)
4. Healthy men	0.83 ± 0.07 (n=16)	—	—	—	—

Statistical significance: 1(b) vs. 1(c), $P < 0.001$; 1(c) vs. 1(e), $P < 0.001$; 2(b) vs. 2(c), $P < 0.05$.

first or second phases of the menstrual cycle, and after the menopause.

It can be seen by inspection of column "a" of Table II that there is no significant difference between the mean values for all subjects studied in Group 1 (normal females) and Group 4 (normal males). Moreover, there is no significant difference in the overall mean values for patients with tumours of the breast (Table II, column "a"; Groups 2 and 3) and normal female subjects (Group 1).

In contrast to the lack of significant differences between the overall mean values just mentioned, when the female Groups 1-3 are separated into first or second phases of the menstrual cycle and post-menopausal sub-groups (Table II, columns "b", "c", "e") then significant differences become evident. There is a considerable increase in the IC_{50} values for healthy women in the second phase of the cycle compared to the first phase; the post-menopausal sub-group (column "e") for normal women gives a mean value that is very similar to that obtained in the first phase of the cycle in pre-menopausal subjects. The stage of the cycle in the subjects studied was calculated on the basis of the time from the onset of menstruation, and, in some normal subjects, was checked by measurements of body temperature and progesterone levels in the plasma. Where the progesterone concentration indicated a failure of ovulation the value for the second phase of the cycle was deleted (3 subjects). In fact where progesterone had not increased in the presumed second phase of the cycle, the IC_{50} value

was in the range typical of first phase samples. The latter result suggests that the change in IC_{50} that is observed between the first and second phases of the cycle is probably a more or less direct consequence of hormonal influences.

The mean value for normal males (Group 4, column "a") corresponds to that found for normal pre-menopausal women in the first phase of the cycle, and for post-menopausal women.

Although the overall difference between the mean values obtained for first and second phases of the menstrual cycle is not so large in patients with tumours of the breast (Groups 2 and 3) as in normal subjects (Group 1), the same phenomenon is observed: a higher value for IC_{50} in the second phase compared to the first phase of the cycle. This difference is statistically significant for the benign tumour group, but is not statistically significant in the malignant tumour group due to a larger variability between patients in Group 3 than Groups 1 and 2. The variation between individual values is clearly shown in the "scatter diagram" (Figure 1), which emphasises the clear differences found between first and second phases of the menstrual cycle in relation to the IC_{50} values for PGI_2 .

The IC_{50} values for the malignant tumour group (Group 3) were also considered in relation to the existence of hypertension or obesity, and to the occurrence of lymph node metastases. Although the number of patients with hypertension was small, and all were post-menopausal, there was a tendency for the IC_{50} values to be increased. No apparent

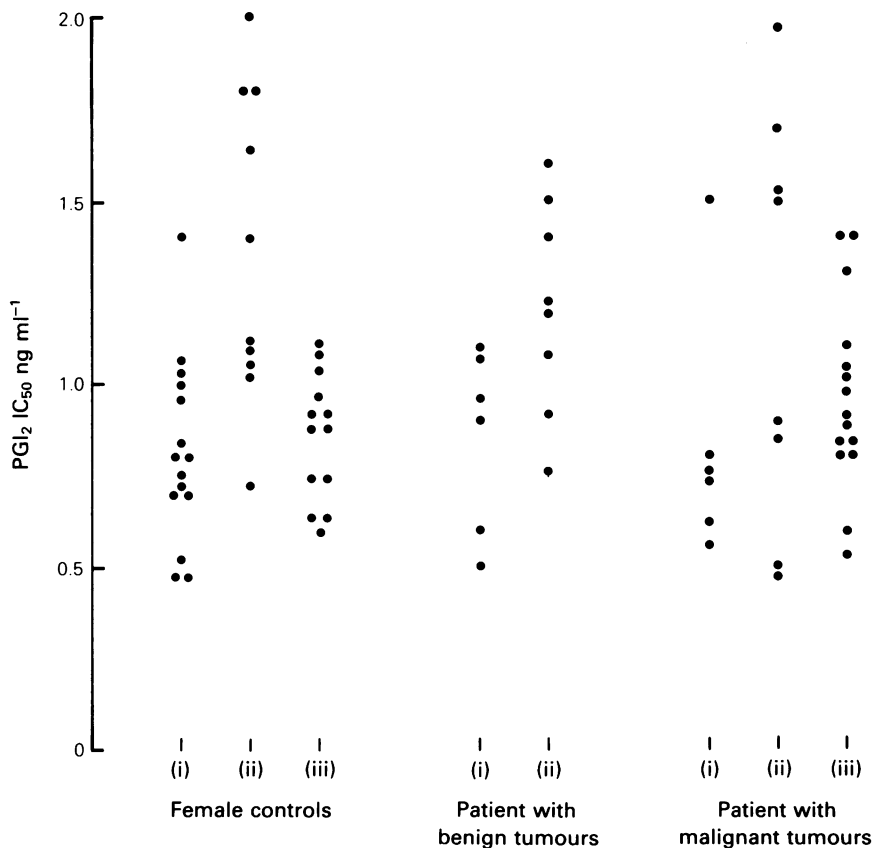


Figure 1 Individual values of IC_{50} for the effect of PGI_2 on ADP-induced platelet aggregation in female control subjects and in patients with benign and malignant tumours of the breast during the first (i) and second (ii) phase of the menstrual cycle, and after the menopause (iii).

change in the IC_{50} occurred in association with obesity. In patients with lymph node metastases, both before or after the menopause, there was no significant change in the IC_{50} values compared to corresponding patients without metastases.

Discussion

It is clear from the results presented in Table II for female subjects that platelet sensitivity to prostacyclin, as reflected by the IC_{50} values, is markedly dependent upon the phase of the menstrual cycle, and that if this is not allowed for then misleading conclusions may ensue.

Although no special attempt was made here to determine the variation, if any, of the IC_{50} with age of women, it is evident from Table II that normal premenopausal women (average age 28 years) have a higher IC_{50} (i.e. the platelets are less sensitive to PGI_2) than normal post-menopausal subjects

(average age 54 years); a decrease of IC_{50} with age has been reported by Sinzinger *et al.* (1981).

The IC_{50} values obtained here for normal men and women are in the same general range of values reported by previous investigators, who did not separate the female subjects into the first and second phases of the menstrual cycle and post-menopause. For example, our overall values for normal females and males are 0.97 ± 0.05 and $0.83 \pm 0.07 \text{ ng ml}^{-1}$ respectively, compared to $0.4 \pm 0.1 \text{ ng ml}^{-1}$ found by Whittle & Moncada (1983), who did not specify the sex of the blood donors, and 0.91 ± 0.07 for females and $0.92 \pm 0.09 \text{ ng ml}^{-1}$ for males aged 41–50 years (Sinzinger *et al.*, 1981).

Our results with blood samples taken from patients with benign tumours of the breast are very similar to the results obtained with normal female subjects (Table II, Group 1 vs. 2). In Group 2 we have not been able to include values for post-menopausal women as fibroadenomas in the post-

menopause are not common (Sinkovics, 1979). The rather smaller difference observed between the first and second phases of the menstrual cycle in the benign tumour group compared to normal (0.86–1.21 compared to 0.81–1.37) may be possibly a reflection of the higher average age in Group 2 (39 ± 6 years) compared to Group 1 (28 ± 4 years).

The results found for healthy women and for women with malignant breast tumours (Table II) are similar to our preliminary findings (Benedetto *et al.*, 1984) with an important proviso that is detailed below. In that preliminary report we concluded from our results obtained on 9 control and 9 malignant samples that the IC_{50} values for the malignant tumour group were smaller than those found in the control samples. However, by chance, our early control samples were mostly from women in the 2nd phase of the cycle (equivalent to Group 1c, Table II), and our early malignant samples were mostly from post-menopausal women (equivalent to Group 3e, Table II). In consequence, our preliminary report compared women of dissimilar hormonal status; our present results are indeed very similar for those two groups of samples (Group 1c vs. Group 3e). However, when women of comparable hormonal status are considered, which is a major point emphasised here, and uncovered as a result of this much larger study, there are no

significant differences between the normal and tumour groups. These results show that platelets prepared from the blood of patients with malignant tumours of the breast are not significantly different in their response to PGI_2 in relation to ADP-induced aggregation. If platelets of cancer patients show enhanced aggregation *in vivo*, as predicted by Honn's hypothesis (Honn 1982; Honn *et al.*, 1983) then other contributory factors should be investigated, such as local concentrations of PGI_2 and TXA_2 , and sensitivity of platelets to TXA_2 .

Finally, we wish to emphasise the importance, in studies of this kind on PGI_2 sensitivity, of clearly separating the sub-groups of patients and normal subjects with different hormonal status; unless this is controlled then important differences between groups under evaluation may be overlooked.

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