# Inhibition of Cancer Cell Stickiness by the Blocking of Platelet Aggregation

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## SUMMARY

**The mechanisms of the early stage of metastasis formation by blood-borne cancer cells is described. Abnormal platelet aggregation, alteration of coagulation and fibrinolysis play an important part.**

**The reducing effect on cancer cell stickiness of dipyridamole, some of its derivative compounds and two other vasodilating agents were investigated in animals. A clinical trial was started 15 months ago with the dipyridamole compound RA 233 in patients with sarcoma of the head and neck region.**


*S. Afr. Med. J.*, **48**, 621 (1974).

The first step in the formation of metastasis is the secure attachment of blood-borne cancer cells to the endothelium of capillaries. In experimental animals, as in humans, the vascular endothelium represents the universal host of — and barrier to — circulating cancer cells.[1] Therefore the interaction between endothelium and cancer cells is a focal point of the metastasis problem.

In 1903 Schmidt[2] was the first to demonstrate cancer cells in the arterioles of lungs of deceased cancer patients; these cells were attached to the vascular wall and surrounded by conglutinated platelets, leucocytes and a dense network of fibrin. These observations were confirmed by other investigators with histological studies of human postmortem material[3,4] and experimental systems.[3,5-7]

Since the cancer cell attachment to the vascular endothelium and the consequent thrombus formation are dynamic events, the pathogenesis of the early stage of metastasis production by blood-borne cancer cells cannot be evaluated by the interpretation of the static morphological findings alone.

Progress was made by viewing the sequence of events in metastasis formation using intravital capillary microscopy. The intravital microcinematographic recordings of Wood *et al.*[1,8] and Gastpar *et al.*[9] are particularly valuable. They demonstrated the fate of intravenously transplanted single ascites cells in V$_2$-carcinoma, Lewis-carcinoma-150, Walker-256-carcinosarcoma and Yoshida-sarcoma in arterioles, capillaries and venules of the ear chamber of rabbits, the mesentery of rats and the peritoneum of mice.

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## HOW CANCER CELLS CAUSE OBSTRUCTION

Cancer cells display a varying tendency to stick to the vascular endothelium. Platelets may aggregate on sticky cancer cells during their circulation. Such a tumour attaches more readily to the endothelium. Once tumour cells are attached, the cross-section of the small vessels is diminished, thus obstructing blood flow. Turbulence results behind the obstruction. Thrombocytes and leucocytes are attracted into this turbulence and preferably aggregate there. Within minutes they become enmeshed in a fibrin clot. In a few hours, the lodged cancer cells are able to penetrate the vessel wall, often following an emigrating lymphocyte.[10] These cancer cells either proliferate in the extravascular tissue or traverse the interstitial space and re-enter the lymphatics, analogous to the continuous recirculation of lymphocytes from the blood back into the lymph, as demonstrated by Gowans.[11]

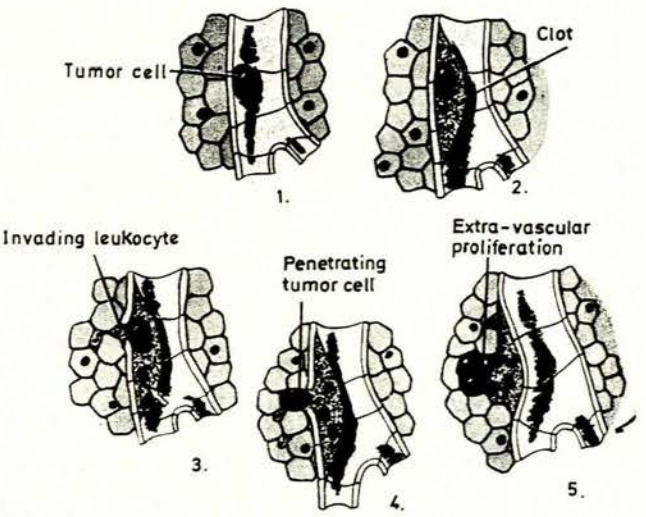


**Fig. 1. Conversion of a tumour cell embolus to a metastasis (Fisher and Fisher[27]).**

The dynamics of the early stage of metastasis formation, first demonstrated by Wood *et al.*[1] by intravital microcinematography, can be classified into three phases (Fig. 1):

1. The attachment of sticky cancer cells to the endothelium.

2. The aggregation of platelets and leucocytes round the attached cancer cells followed by the formation of a fibrin clot.

3. The penetration of the vessel wall by the arrested cancer cells and extravascular proliferation.

The initial adherence of embolic cancer cells to intact vascular endothelium observed *in vivo* is independent of capillary diameter, rate of the blood flow or vasomotor activity.[1,12] The labile capillaries do not function as simple mechanical filters of embolic cancer cells. Rather, the lodgement of these circulating cancer cells on the endothelium depends mainly on their 'stickiness',[13] a specific indigenous physicochemical property of the cell surface[14] related to its thromboplastic activity.[1,15-17] Its influence on metastasis production has been demonstrated with particular distinctness in various strains of rat ascites hepatomata by Kojima and Sakai.[18] The strain-specific cellular stickiness *in vitro* was closely related to the strain-specific frequency pattern of pulmonary metastasis. Furthermore, it was found that the stickiness of tumour cells decreased significantly by *in vitro* pretreatment with trypsin and heparin.[16]

## FACTORS INFLUENCING METASTASIS FORMATION

Since the impressive studies of Lawrence *et al.*[17,19] and Cliffton and Grossi,[20] evidence has accumulated showing that anticoagulants and fibrinolytic drugs interfere with the initial adherence of cancer cells to the vascular endothelium and their enmeshment in a fibrin clot. These drugs reduce the incidence of metastasis production of some experimental tumours.[5,21,22] Furthermore, it might be conjectured that anticoagulants promote the traversing of cancer cells via the interstitial route, at the same time preventing their extravascular proliferation.[23]

In contrast to the anticoagulant and fibrinolytic agents which exert a protective effect against the formation of metastases from blood-borne cancer cells by means of reducing their stickiness, there are certain experimental factors which enhance the stickiness of cancer cells, leucocytes, thrombocytes or endothelium, and/or produce potential hypercoagulability. Examples are stress, cortisone treatment, trauma, hyperlipaemia, antifibrinolytic agents, endotoxin, radiotherapy and sublethal doses of cytostatic drugs.[5,21,22] These agents generally may favour metastasis production from embolic tumour cells, as demonstrated in Fig. 2. On the left are noted factors that may enhance this event. On the right are listed agents that have been investigated for their ability to reduce the incidence of metastasis production in certain experimental tumours.

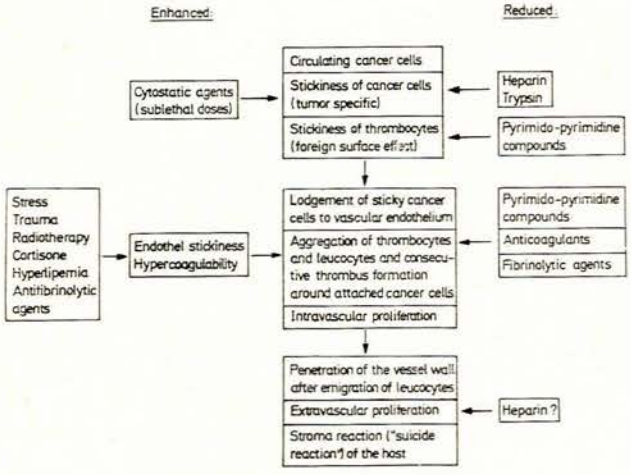


**Fig. 2. Proposed scheme for metastasis formation from blood-borne cancer cells with factors influencing this event (Gastpar[20]).**

## DIPYRIDAMOLE

Since thrombocytes play an important role in the mechanism of tumour cell lodgement and subsequent thrombus formation, we assumed that substances which block aggregation of thrombocytes also assist in the inhibition of lodgement of tumour cells.[10] Dipyridamole (Fig. 3) has

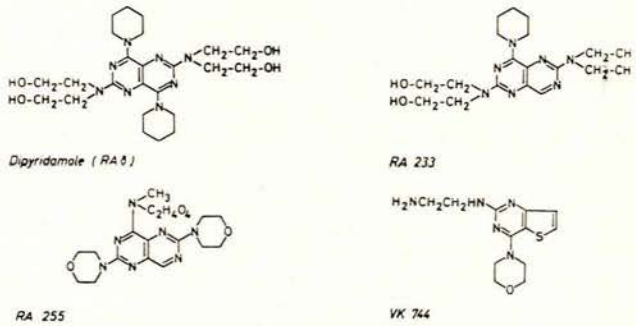


**Fig. 3. Chemical structures of dipyridamole (RA 8) and the pyrimido-pyrimidine derivatives RA 233, RA 255 and VK 744 (Gastpar[25]).**

been found to reduce the spontaneous aggregation of platelets,[24] to decrease thrombus formation in response to vascular injury in rabbits,[25] to alter the electrophoretic mobility of platelets in response to norepinephrine,[26] and to diminish platelet adhesiveness in patients with coronary artery disease[27,28] and renal disease.[29,30] Some other pyrimido-pyrimidine compounds have also been shown to influence the platelet behaviour *in vitro* and *in vivo*.[31-34] Some of the effects of these drugs on platelet aggregation, bleeding time, blood pressure and thrombus formation are shown in Table I.

## NEW PYRIMIDO-PYRIMIDINE DERIVATIVES

We have determined the influence of some new pyrimido-pyrimidine derivatives on the stickiness of circulating tumour cells by intravital microscopy in the mesentery of rats.[10,35] The mesentery of Wistar rats of 150 - 200 g body

**TABLE I. ACUTE TOXICITY AND THE EFFECTS OF 4 PYRIMIDO-PYRIMIDINE COMPOUNDS ON ADP- AND COLLAGEN-INDUCED AGGREGATION (BORN TEST) AND RETENTION OF PLATELETS (MORRIS TEST), BLEEDING TIME, THROMBUS FORMATION AND BLOOD PRESSURE[38]**

| | | Ra 8 | Ra 233 | VK 744 | Ra 255 |
|---|---|---|---|---|---|
| Born test | ADP | | | | |
| | 1st phase $5 \times 10^{-5}$mol. | 0 | 51% | 27% | 21% |
| | Oral | 0 | 70% | 65% | 49% |
| | Collagen 50% inhibition | $5 \times 10^{-4}$ | $3 \times 10^{-5}$ | $6 \times 10^{-5}$ | $5 \times 10^{-5}$ |
| Retention test (Morris) | $3 \times 10^{-5}$mol | 48% | 39% | 76% | 31% |
| | $10^{-5}$mol | 15% | 15% | 35% | 10% |
| | $5 \times 10^{-6}$mol | 0 | 0 | 0 | |
| Bleeding time prolongation | 1 hour after 10 mg/kg oral | 8,9 min | 7,8 min | 11,4 min | 11,9 min |
| | control: 4,0 min ±0,42 | ±0,78 | ±0,46 | ±0,64 | ±0,36 |
| Thrombus formation inhibition | 1 hour after 10 mg/kg oral | 46% | 41% (20 mg) | 62% | 72% |
| BP reduction (2 mg/kg IV) | mmHg | −52 | −23 | −8 | −32 |
| | Duration (min) | 58 | 14 | 1 | 5 |
| Acute toxicity mice $LD_{50}$ | IV | 150 mg/kg | 148 mg/kg | 115 mg/kg | 216 mg/kg |
| | Oral | >2 g/kg | 465 mg/kg | 680 mg/kg | >3 g/kg |

mass was dissected after anaesthesia with chloral hydrate. Two millilitres of a suspension containing $1 \times 10^9$ fluorochromated Walker-256-carcinosarcoma cells were slowly injected into a polyethylene catheter inserted into the jugular vein. Solutions of various doses of dipyridamole (RA 8) and the pyrimido-pyrimidine compounds RA 233, RA 255 and VK 744 were injected into the catheter 5 minutes before tumour cell transplantation. The platelet count in the venous blood of the animals was determined 10 minutes before and 20 minutes after tumour cell transplantation.

As demonstrated in Table II, within 5 - 10 minutes after the infusion, fatal tumour cell embolism of the lungs was observed in 60% of the control animals (column 4). This was due to the blockade of pulmonary capillaries and arterioles by lodged and clumped tumour cells, which in some instances produced occlusion of a complete pulmonary segment. This lethal rate was diminished to 27% by intravenous injection of 10 mg/kg of dipyridamole and to zero by RA 233, RA 255 and VK 744.

The dose-response relationships of these substances on fatal tumour cell embolism of the lungs was quantified by a probit analysis[36] as illustrated in Fig. 4. The significance of difference between the regression lines was

**TABLE II. EFFECT OF 4 PYRIMIDO-PYRIMIDINE DERIVATIVES ON PULMONARY TUMOUR CELL EMBOLISM IN RATS AFTER INTRAVENOUS TRANSPLANTATION OF $1 \times 10^5$ WALKER-256-CARCINOSARCOMA CELLS VIA JUGULAR VEIN[33]**

| Substance | Dose | Mortality | Mortality (%) |
|---|---|---|---|
| Controls | | 36/60 | 60 |
| RA 8† | 3 mg/kg | 19/40 | 48 |
| RA 8† | 6 mg/kg | 14/40 | 35 |
| RA 8† | 10 mg/kg | 8/30 | 27 |
| RA 233 | 3 mg/kg | 12/40 | 30 |
| RA 233 | 6 mg/kg | 3/30 | 10 |
| RA 233 | 10 mg/kg | 0/20 | — |
| RA 255 | 3 mg/kg | 10/40 | 25 |
| RA 255 | 6 mg/kg | 2/40 | 5 |
| RA 255 | 10 mg/kg | 0/40 | — |
| VK 744 | 3 mg/kg | 5/30 | 17 |
| VK 744 | 6 mg/kg | 1/30 | 3 |
| VK 744 | 10 mg/kg | 0/30 | — |

† Dipyridamole (Persantin).

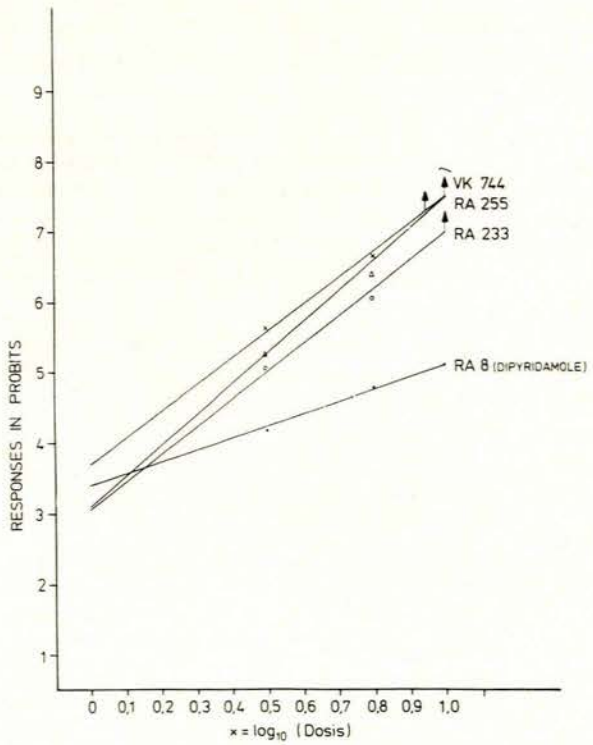


**Fig. 4. Probit regression lines for effect of 4 pyrimido-pyrimidine compounds on tumour cell embolism mortality in rats.**

**TABLE III. EFFECT OF 4 PYRIMIDO-PYRIMIDINE COMPOUNDS ON THE PLATELET COUNT IN SURVIVING RATS AFTER INTRAVENOUS TRANSPLANTATION OF 1 × $10^5$ WALKER-256-CARCINOSARCOMA CELLS[22]**

| Substance, dose | No. of survivors | Platelet count before trial: Mean value | Platelet count before trial: Standard deviation | Platelet count before trial: Standard error | Platelet count after trial: Mean value | Platelet count after trial: Standard deviation | Platelet count after trial: Standard error | Reduction (%) |
|---|---|---|---|---|---|---|---|---|
| **Controls** | **24** | **702,1** | **56,31** | **11,49** | **169,2** | **74,45** | **15,19** | **−75,9** |
| **RA 8†** | | | | | | | | |
| **3 mg/kg** | **21** | **724,1** | **79,36** | **17,32** | **224,5** | **92,15** | **20,11** | **−69,0** |
| **10 mg/kg** | **22** | **729,7** | **55,98** | **11,93** | **297,8** | **74,50** | **15,88** | **−59,2** |
| **RA 233** | | | | | | | | |
| **3 mg/kg** | **28** | **705,6** | **42,94** | **8,11** | **273,5** | **71,02** | **13,42** | **−61,2** |
| **10 mg/kg** | **20** | **708,6** | **39,60** | **8,86** | **615,0** | **55,10** | **12,32** | **−14,6** |
| **RA 255** | | | | | | | | |
| **3 mg/kg** | **30** | **708,2** | **33,05** | **6,04** | **289,9** | **57,48** | **10,48** | **−59,1** |
| **10 mg/kg** | **40** | **703,6** | **28,14** | **4,45** | **696,0** | **39,14** | **6,19** | **− 1,1** |
| **VK 744** | | | | | | | | |
| **3 mg/kg** | **25** | **709,0** | **36,21** | **7,24** | **512,4** | **31,77** | **6,35** | **−27,7** |
| **10 mg/kg** | **30** | **693,9** | **30,17** | **5,50** | **687,9** | **34,22** | **6,24** | **− 0,9** |

† Dipyridamole (Persantin).

examined. In contrast to normal probit analysis the portion of surviving animal represent percentage points of the diagram; the arrows indicate 100% response rates.

The platelet count in the venous blood of untreated animals and those treated with low doses was significantly reduced compared with animals who received higher doses of the pyrimido-pyrimidine derivatives. Table III presents mean values, standard deviations, standard errors and percentage reduction for the different dose groups and test substances. The summarising statistics, however, must be interpreted with some caution, because the samples from which they were calculated, in most instances, are selected from death cases. Nevertheless, a trend may be recognised, and the standard errors will give some hint concerning the significance of the differences. This may be interpreted by a protection of the rats sufficiently treated against lethal tumour cell embolism of the lungs, effected by an inhibition of the platelet aggregation.

The criterion for the reaction of the test substances on the thrombocyte adhesiveness and aggregation was the number of infused tumour cells that could be detected in a certain area of 1 $cm^2$, showing adherence to the vascular endothelium during a period of 30 minutes of observation. Table IV notes the number of the *adhering tumour cells.*

**TABLE IV. THE EFFECT OF 4 PYRIMIDO-PYRIMIDINE DERIVATIVES ON THE INITIAL LATENT ADHERENCE OF TUMOUR CELLS TO VASCULAR ENDOTHELIUM IN THE MESENTERY OF RATS AFTER INTRAVENOUS TRANSPLANTATION OF 1 × $10^6$ WALKER-256-CARCINOSARCOMA CELLS VIA JUGULAR VEIN[33]**

| Substance | Dose | Variation cells/animal | Mean values cells/animal | No. of animals |
|---|---|---|---|---|
| **Controls** | | **24 - 96** | **57** | **24** |
| **RA 8†** | **3 mg/kg** | **20 - 98** | **53** | **21** |
| **RA 8†** | **6 mg/kg** | **13 - 67** | **45** | **26** |
| **RA 8†** | **10 mg/kg** | **10 - 55** | **41** | **22** |
| **RA 233** | **3 mg/kg** | **14 - 71** | **41** | **28** |
| **RA 233** | **6 mg/kg** | **11 - 43** | **28** | **27** |
| **RA 233** | **10 mg/kg** | **2 - 31** | **18** | **20** |
| **RA 255** | **3 mg/kg** | **10 - 54** | **35** | **30** |
| **RA 255** | **6 mg/kg** | **8 - 37** | **21** | **38** |
| **RA 255** | **10 mg/kg** | **1 - 22** | **12** | **40** |
| **VK 744** | **3 mg/kg** | **8 - 46** | **30** | **25** |
| **VK 744** | **6 mg/kg** | **3 - 21** | **13** | **29** |
| **VK 744** | **10 mg/kg** | **0 - 11** | **7** | **30** |

† Dipyridamole (Persantin).

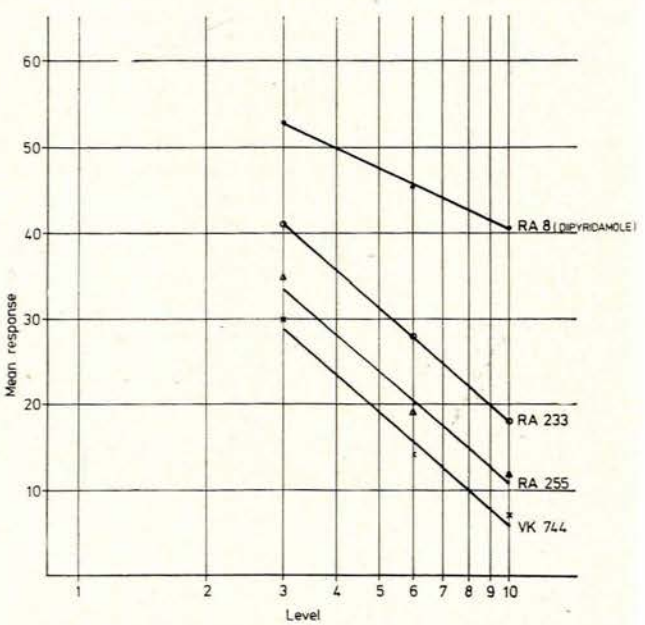


**Fig. 5. Dose-response relationship for the effect of RA 8, RA 233, RA 255 and VK 744 on latent cancer cell stickiness in the mesentery of rats.**

In the controls the mean value was 57 cells per animal, specified in column 4.

Dipyridamole and the other tested pyrimido-pyrimidine derivatives caused a marked reduction of tumour cell adherence. The compounds RA 233, RA 255 and VK 744 were the most potent ones.

The dose-response relationships for cancer cell stickiness data were investigated by means of variance analysis (Fig. 5). Although the distribution of the data of the highest dosage groups may not meet the criteria of this analysis, it will serve to introduce only a small bias into the results because of the relative inaccuracy of the analysis of variance technique. The dose-response curves are displayed graphically by plotting the mean response against the logarithm of the stimulus level. It may be seen that the differences among the dosage groups for RA 8, RA 233, RA 255 and VK 744 are highly significant. These differences may be explained by a linear trend component, so that linear dose-response relationship can be assumed. The order of effectiveness of the test substances was the same as in the mortality from pulmonary embolism.

## SH 869, BENCYCLANE AND BL 191

Recent unpublished experiments with SH 869, a new pyrimido-pyrimidine derivative, as well as with bencyclane-hydrogen fumarate and the methylxanthine derivative BL 191, two vasodilating substances, showed that these substances are also able to reduce the pulmonary embolism mortality, to hinder platelet aggregation on circulating sticky cancer cells, and to inhibit their attachment to the endothelium.

As demonstrated in Table V, the rate of lethal tumour cell embolism was diminished from 62,5% to 17% by 12 mg/kg BL 191, to 13% by 12 mg/kg bencyclane and 4 mg/kg SH 869 respectively administered intravenously, and to zero by 8 mg/kg SH 869 administered intragastrically. The dose-response curves for the effect of these substances on fatal tumour cell embolism also were quantified by a probit analysis, as illustrated in Fig. 6.

**TABLE V. EFFECT OF BENCYCLANE, BL 191 AND SH 869 ON PULMONARY TUMOUR CELL EMBOLISM IN RATS AFTER INTRAVENOUS TRANSPLANTATION OF 1 × $10^5$ WALKER-256-CARCINOSARCOMA CELLS VIA JUGULAR VEIN**

| Substance, dose | Mortality | Mortality rate (%) |
|---|---|---|
| **Controls** | **25/40** | **62,5** |
| **Bencyclane*** | | |
| **3 mg/kg** | **14/30** | **46,7** |
| **6 mg/kg** | **9/30** | **30,0** |
| **12 mg/kg** | **4/30** | **13,3** |
| **BL 191†** | | |
| **3 mg/kg** | **14/30** | **46,7** |
| **6 mg/kg** | **10/30** | **33,3** |
| **12 mg/kg** | **5/30** | **16,7** |
| **SH 869 intragastric‡** | | |
| **2 mg/kg** | **6/30** | **20,0** |
| **4 mg/kg** | **2/30** | **6,7** |
| **8 mg/kg** | **0/30** | **—** |
| **SH 869 intravenous‡** | | |
| **1 mg/kg** | **15/30** | **50,0** |
| **2 mg/kg** | **8/30** | **28,9** |
| **4 mg/kg** | **4/30** | **13,3** |

* N-(3-(1bencyl-cycloheptyl-oxy)propyl)-N.N.-dimethyl-ammonium hydrogenfumarate.
† 3,7-dimethyl-1-(5-oxo-hexyl)-xanthine.
‡ 2-piperacinyl-4-thiomorpholino-pyrido(3.2-d)pyrimidine-sulphate-trihydrate.

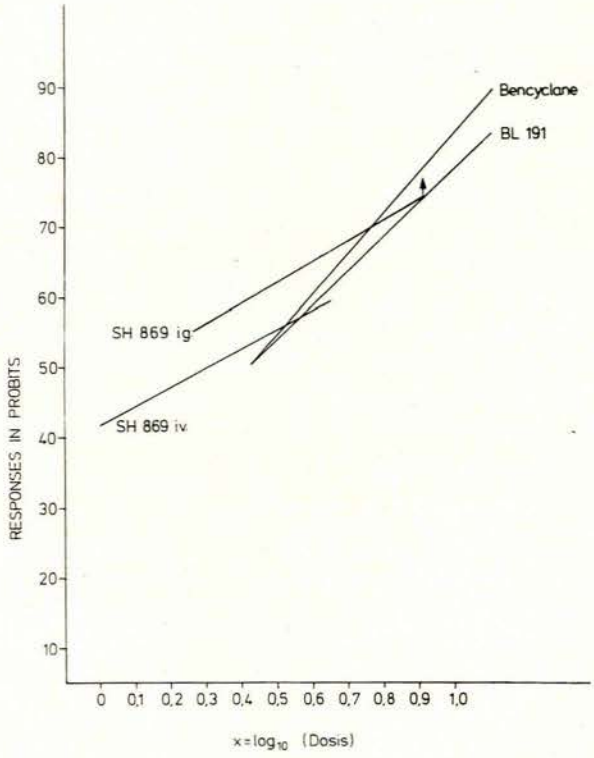


**Fig. 6. Probit regression lines for the effect of bencyclane, BL 191, and SH 869 on tumour cell embolism mortality in rats.**

The platelet count in the venous blood of untreated animals and those treated with insufficient doses was significantly reduced compared with animals which received the highest dose groups of the test substances (Table VI). The most effective protection of the platelet count against reduction resulted after intragastric administration of 8 mg/kg SH 869.

The number of adhering tumour cells is listed in Table VII. In the controls the mean value was 54 cells per animal, specified in column 3. Bencyclane and BL 191 caused a marked reduction of tumour cell adherence, to a mean of 29 and 24 cells respectively per animal. With intravenous SH 869 the number of lodged tumour cells dropped to a mean of 10 cells and to a mean of only 1 cell per animal after intragastric administration.

**TABLE VI. EFFECT OF SH 869 (INTRAVENOUS AND INTRAGASTRIC ADMINISTRATION), BENCYCLANE AND BL 191 ON THE PLATELET COUNT IN SURVIVING RATS AFTER INTRAVENOUS TRANSPLANTATION OF 1 × $10^6$ WALKER-256-CARCINOSARCOMA CELLS**

| | | Platelet count before trial | | | Platelet count after trial | | | |
|---|---|---|---|---|---|---|---|---|
| Substance, dose | No. of survivors | Mean value | Standard deviation | Standard error | Mean value | Standard deviation | Standard error | Reduction (%) |
| **Controls** | 15 | 698 | 16 | 4 | 165 | 20 | 5 | −76,4 |
| **SH 869 intravenous** | | | | | | | | |
| 1 mg/kg | 15 | 674 | 21 | 5 | 298 | 18 | 5 | −55,8 |
| 4 mg/kg | 26 | 682 | 26 | 5 | 579 | 30 | 6 | −15,1 |
| **SH 869 intragastric** | | | | | | | | |
| 2 mg/kg | 24 | 703 | 20 | 4 | 541 | 31 | 6 | −23,1 |
| 8 mg/kg | 30 | 692 | 23 | 4 | 688 | 21 | 4 | − 0,6 |
| **Bencyclane** | | | | | | | | |
| 3 mg/kg | 16 | 719 | 18 | 3 | 247 | 21 | 8 | −65,6 |
| 12 mg/kg | 26 | 709 | 24 | 3 | 324 | 15 | 5 | −52,9 |
| **BL 191** | | | | | | | | |
| 3 mg/kg | 16 | 702 | 14 | 2 | 235 | 15 | 6 | −66,5 |
| 12 mg/kg | 25 | 683 | 16 | 2 | 356 | 29 | 8 | −48,0 |

**TABLE VII. EFFECT OF BENCYCLANE, BL 191 AND SH 869 ON THE INITIAL ADHERENCE OF TUMOUR CELLS TO VASCULAR ENDOTHELIUM IN THE MESENTERY OF RATS AFTER INTRAVENOUS TRANSPLANTATION OF 1 × $10^6$ WALKER-256-CARCINOSARCOMA CELLS VIA JUGULAR VEIN**

| Substance, dose | Variation cells/animal | Mean values cells/animal | No. of animals |
|---|---|---|---|
| **Controls** | 29 - 81 | 54 | 15 |
| **Bencyclane** | | | |
| 3 mg/kg | 20 - 68 | 49 | 16 |
| 6 mg/kg | 17 - 51 | 40 | 21 |
| 12 mg/kg | 10 - 45 | 29 | 26 |
| **BL 191** | | | |
| 3 mg/kg | 23 - 61 | 47 | 16 |
| 6 mg/kg | 16 - 53 | 36 | 20 |
| 12 mg/kg | 12 - 41 | 24 | 25 |
| **SH 869 intragastric** | | | |
| 2 mg/kg | 6 - 21 | 13 | 24 |
| 4 mg/kg | 3 - 14 | 7 | 28 |
| 8 mg/kg | 0 - 4 | 1 | 30 |
| **SH 869 intravenous** | | | |
| 1 mg/kg | 21 - 52 | 44 | 15 |
| 2 mg/kg | 9 - 34 | 21 | 22 |
| 4 mg/kg | 5 - 19 | 10 | 26 |

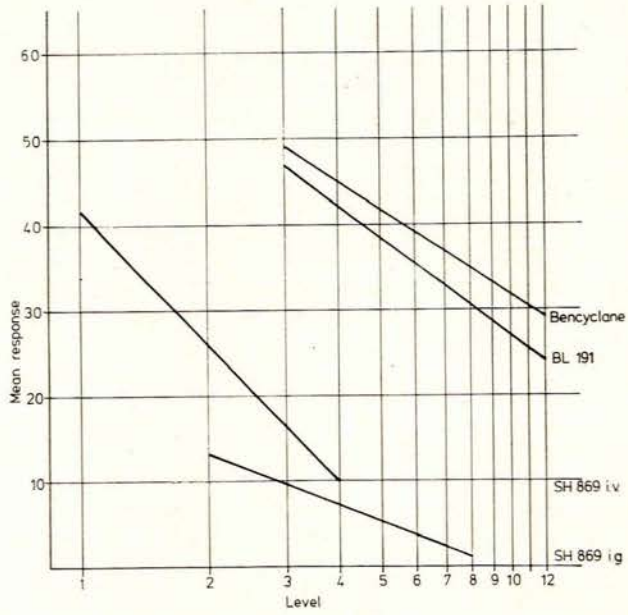


**Fig. 7. Dose-response relationship for the effect of bencyclane, BL 191 and SH 869 on latent cancer cell stickiness in the mesentery of rats.**

The dose-response relationship for cancer cell stickiness, investigated by means of variance analysis, is demonstrated in Fig. 7. It may be seen that the differences among the dosage groups are highly significant, so that linear dose-response relationship for all three substances can be assumed.

It is interesting to note that the new pyrimido-pyrimidine compound SH 869 was more efficacious when administered intragastrically than when administered intravenously. This may be explained by a high absorption rate and a quick transformation into a more effective metabolite.

Bencyclane, BL 191 and the pyrimido-pyrimidine derivatives used, especially the compounds RA 233, RA 255, VK 744 and SH 869, were able to interfere with the dynamic interaction between sticky tumour cells and vascular endothelium, just as heparin and fibrinolysin do, but at an earlier stage. They inhibited platelet adhesiveness and aggregation to circulating sticky tumour cells and significantly inhibited their attachment to the endothelium. Also, these compounds hindered the platelet aggregation round adherent cancer cells and blocked subsequent thrombus

formation. Further proof of this is that some of these substances prevented lethal pulmonary tumour cell embolism which occurred in 60 - 62,5% of the controls.

It remains to be seen whether other animal tumours can be affected in the same manner. It should also be determined whether these substances can significantly reduce the rate of uptake and the frequency pattern of tumour metastasis.

## CLINICAL APPLICATION

On the basis of our experimental data and because the clinical and toxicological conditions of the pyrimido-pyrimidine compound RA 233 allowed a daily long-term therapy,* we started a controlled clinical study on metastasis prophylaxis 15 months ago. We used this substance in 25 patients with sarcoma of the head and neck region (after surgery and radiation therapy) in a daily dosage of 3 × 250 mg and 3 × 500 mg respectively (Table VIII). Since this was not a controlled study, results should be treated with some reservation. It is, however, my experience that metastases or new tumours do occur within one year in 15 - 35% of all patients. By the same token, I realise that the number of patients in this group is small and the time of medication and observation is short, but until now none of our patients has developed clinical signs of a local recurrence or of a metastasis. The clinical tolerance of RA 233 is good and no side-effects appeared. This is encouraging as an effective beginning of metastasis prophylaxis in cancer.

**TABLE VIII. METASTASIS PROPHYLAXIS WITH DAILY LONG-TERM THERAPY OF THE PYRIMIDO-PYRIMIDINE COMPOUND RA 233 IN 25 PATIENTS WITH SARCOMA OF THE HEAD AND NECK†**

| No. of patients | Duration of medication with RA 233 (mo.) | No. of patients with daily dosage of | |
|---|---|---|---|
| | | 3 × 250 mg | 3 × 500 mg |
| 3 | 15 | 2 | 1 |
| 2 | 12 | — | 2 |
| 2 | 11 | 1 | 1 |
| 2 | 9 | 1 | 1 |
| 2 | 8 | — | 2 |
| 5 | 7 | 4 | 1 |
| 4 | 6 | — | 4 |
| 2 | 3 | — | 2 |
| 2 | 2 | — | 2 |
| 1 (25) | 1 | — (8) | 1 (17) |

† 12 reticulum cell sarcoma, 3 lymphosarcoma, 3 Hodgkin's sarcoma, 2 melanosarcoma, 2 angioplastic sarcoma, 1 anaplastic sarcoma, 1 spinocellular sarcoma, 1 rhabdomyosarcoma.

*Oral lethal doses in guinea pigs and rats could not be assessed; they are in excess of 3 g/kg body mass. In mice, oral $LD_{50}$ was 465 mg/kg body mass Chronic toxicity was studied in rats and dogs. Pathological alterations due to drug administration could not be demonstrated. Teratogenic investigations with increasing doses in rats and dogs showed negative results.

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