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The Influence of Antiserotonin Treatment with Ketanserin on the Pulmonary Lodgement of Circulating Tumour Cells in Normal and Traumatized Rats*

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Abstract—*As shown in earlier studies, the lodgement of circulating tumour cells in the lungs is reduced by thrombocytopenia in both normal and traumatized rats. Other experiments have shown that thrombocytopenia and antiserotonin treatment with ketanserin, which has a selective effect on 5-HT_2 receptors, decrease the hepatic lodgement of intraportally injected tumour cells. The present studies show that treatment with ketanserin also reduces the lodgement of i.v.-injected tumour cells in the lungs in both normal and traumatized rats.*

INTRODUCTION

IT WAS found in previous studies that the lodgement of intravenously injected tumour cells in the lungs of rats was significantly increased by trauma [1], while thrombocytopenia reduced lodgement and also neutralized the enhancing effect of trauma on tumour cell lodgement [2]. The influence of platelets on the lodgement process was further investigated in two recent studies in the rat liver [3, 4]. A combination of vital fluorescence microscopy, electron microscopy and isotope measurements was used to analyse the lodgement of intraportally injected tumour cells. Thrombocytopenia had no effect on the initial arrest of circulating tumour cells but caused a significant reduction in the number of lodged tumour cells as measured 3 hr after tumour cell injection. Similar results were obtained with ketanserin (Janssen Pharmaceutica, Belgium), a recently available specific serotonin antagonist with selective effect on 5-hydroxytryptamine$_2$ (5-HT_2) receptors.

The aim of the present study was to test the effect of antiserotonin treatment on the pulmonary lodgement of intravenously injected tumour cells in normal and traumatized rats.

MATERIALS AND METHODS

Animals

Hooded rats of the Lister strain, weighing about 200 g, were used. Rats of the same age and sex were used in each individual experiment.

Tumour

The tumour was a syngeneic, transplantable methylcholanthrene-induced fibrosarcoma, which was originally received from the Chester Beatty Research Institute, Sutton, Surrey, U.K. From this tumour a cell suspension was prepared as described by Ivarsson and Rudenstam [5].

Trauma

Bilateral femoral crush fractures were inflicted by pliers under ether anaesthesia. This type of trauma has been used in earlier studies and increased both tumour cell lodgement and metastasis formation [1, 6, 7].

Antiserotonin treatment

Ketanserin was kindly supplied by Janssen Pharmaceutica, Beerse, Belgium. The rats were injected subcutaneously with 0.6 mg/kg body wt. According to the manufacturer this dose causes an antiserotonin effect lasting for at least 5 hr.

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Labelling procedure

The tumour cells were labelled with [^{125}I]-5-iodo-2-deoxyuridine according to Fidler [8]. The labelling procedure and radioactivity measurements have been described in detail in an earlier paper [1].

Statistical methods

The statistical analyses were performed with Student's t test and Wilcoxon's rank sum test.

Experimental procedures

(A) Twenty animals were randomly divided into two equal groups: group 1: control animals injected with only tumour cells; group 2: animals injected subcutaneously with ketanserin 0.6 mg/kg body wt. 1 hr before tumour cell injection. Each animal received 4×10^5 living tumour cells through a tail vein. The viability of the tumour cell suspension was 85%. All animals were killed 4 hr after tumour cell injection by exsanguination through the aorta under ether anaesthesia. The lungs were removed, washed in tap water and put into glass tubes for radioactivity measurements. The amount of ^{125}I in the lungs and in the injected number of tumour cells was determined as count/min. The number of labelled tumour cells in the lungs could then be calculated.

(B) Thirty animals were randomly divided into three equal groups: group I: control animals injected with only tumour cells; group 2: animals traumatized 2 hr before tumour cell injection; group 3: animals traumatized 2 hr before tumour cell injection and injected subcutaneously with ketanserin 0.6 mg/kg body wt 1 hr before tumour cell injection. Each animal received 4×10^5 living tumour cells through a tail vein. The viability of the tumour cell suspension was 90%. All animals were killed 4 hr after tumour cell injection and the same procedures as in experiment A were followed.

RESULTS

Antiserotonin treatment with ketanserin significantly reduced tumour cell lodgement in the lungs (Table 1). In addition, ketanserin counteracted the enhancing effect of trauma on tumour cell lodgement, but the effect of trauma was not completely neutralized. Thus tumour cell lodgement in antiserotonin-treated and traumatized animals was significantly lower than after trauma alone, but still significantly higher than in the controls (Table 2).

Table 1. Influence of ketanserin treatment on tumour cell lodgement in the 4 hr after i.v. cell injection

Procedure	No. of animals	Mean No. of tumour cells in the lungs
Control	10	18092 (S.D. 2175)
Ketanserin	10	14897* (S.D. 1309)

The injected No. of tumour cells was 4×10^5.
*Significant difference from control at $P < 0.001$ (Student) and $P < 0.01$ (Wilcoxon).

Table 2. Influence of trauma and ketanserin treatment on tumour cell lodgement in the lungs 4 hr after i.v. tumour cell injection

Procedure	No. of animals	Mean No. of tumour cells in the lungs
Control	10	23033 (S.D. 4969)
Trauma	10	43172* (S.D. 6606)
Trauma + ketanserin	10	31217†‡ (S.D. 4881)

The injected No. of tumour cells was 4×10^5.
*Significant difference from control at $P < 0.001$ (Student) and $P < 0.01$ (Wilcoxon).
†Significant difference from control at $P < 0.01$ (Student) and $P < 0.01$ (Wilcoxon).
‡Significant difference from trauma at $P < 0.001$ (Student) and $P < 0.01$ (Wilcoxon).

DISCUSSION

While there is no experimental evidence to support the concept that trauma can initiate a primary tumour [9], there are many experimental investigations indicating that trauma may increase metastasis formation [6, 7, 10, 11].

During the last decade evidence has accumulated which indicates possible interactions between the dissemination of cancer cells and the hemostatic system [12]. The connection between trauma and the coagulation system is well known and it seems that activation of the hemostatic system could be one of the mechanisms underlying the enhancing effect of trauma on metastasis formation.

Ivarsson [7] found that trauma increased pulmonary metastasis formation in normal and defibrinogenated but not in thrombocytopenic rats after intravenous tumour cell injection. On the basis of these results Ivarsson concluded that a platelet reaction was necessary for the increase of metastases after trauma and that the effect could be due to an increased formation of platelet aggregates, causing a prolonged lodgement of more tumour cells. This theory gained support by a subsequent investigation, which showed that thrombocytopenia significantly reduced the pulmonary lodgement of i.v. injected tumour cells in both normal and traumatized rats [2]. Reduction of tumour cell lodgement in thrombocytopenic animals has also been reported by Gasic *et al.* [13]. Although an effect of platelets on the lodgement process seems clearly demonstrated, the mech-

anisms underlying the platelet involvement still remain hypothetical.

Microthrombus formation has been suggested to be of importance for the lodgement of circulating tumour cells and metastasis formation [7, 14]. Several investigators, using qualitative [15] as well as quantitative [16] methods, claim, however, that the microthrombus formation is not of primary importance for any of the processes. In addition, in two recent studies on tumour cell lodgement in the liver after intraportal injection we found no evidence that microthrombi should be of primary importance for tumour cell lodgement [3, 4]. Instead, it was suggested that platelets influence the lodgement process by some biochemical action, possibly by release of serotonin. This theory was tested by pretreatment of the animals with ketanserin, a recently available specific antiserotonin compound with selective action on serotonin-2 (5 HT_2) receptors. Ketanserin was found to cause a significant reduction of the lodgement of intraportally injected tumour cells, although the reduction was not as marked as that in thrombocytopenic animals [4]. This investigation was designed to allow studies on the effect of ketanserin also in association with trauma. The results show that ketanserin reduces the pulmonary lodgement of i.v. injected tumour cells in both normal and traumatized animals. When compared with previous results on thrombocytopenia [2], the effect of ketanserin in this study is also less marked than that of thrombocytopenia, which completely neutralized the enhancing effect of trauma on tumour cell lodgement.

In a previous report [4] we discussed some possible explanations of the effect of serotonin on tumour cell lodgement in normal animals. All the proposed mechanisms for serotonin interaction with tumour cell lodgement were based on the concept that serotonin in some way promotes tumour cell survival. The effect could be in the form of direct protection of the tumour cells from injury, a phenomenon which has been observed for cultured fibroblasts incubated with serotonin [17]. The effect could also be indirect, i.e. serotonin might inhibit the phagocytosis of tumour cells by, for example, leukocytes. Such an action of serotonin is supported by studies on the phagocytic properties of protozoa [18]. Another indirect mechanism by which serotonin might enhance tumour cell survival is through an increase of vascular permeability [19], thereby possibly facilitating the escape of tumour cells from the blood stream into the extravascular space. An extravascular location is considered to be more favourable for tumour cell survival than the intravascular compartment, which has been described as a hostile environment for lodged tumour cells [20].

The same mechanisms may also be applicable to the results in this study. Thus the enhanced tumour cell lodgement after trauma could be explained by an increased release of serotonin as a consequence of a general platelet activation which always follows after trauma [21].

It must be pointed out, however, that ketanserin, in contrast to thrombocytopenia, does not completely neutralize the effect of trauma on tumour cell lodgement. This difference could be a matter of effectiveness, i.e. the given dose of ketanserin might be too small to block all serotonin-2 receptors in a post-traumatic situation. Other mechanisms besides serotonin release might also be considered to account for the platelet action on the lodgement of tumour cells. Obviously, further experiments are required for a complete understanding of the role of platelets in the lodgement of circulating tumour cells.

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