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Urokinase Inhibitor in Patients with Bladder Cancer*

H. Hisazumi, K. Naito, T. Misaki, and S. Kosaka

Department of Urology, School of Medicine, Kanazawa University, Kanazawa-city, Japan

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Summary. Plasma and tissue inhibitory activities were measured by the fibrin plate method in 33 plasma samples and 25 cancerous tissue specimens from 48 patients with bladder cancer, and were compared with those of normal plasma and bladder tissues, respectively. Both the inhibitory activities were significantly higher in the patients than in normal subjects. In addition, they were significantly higher in patients with cancer of high grades (Grades 2, 3 and 4) and high stages (Stages B, C and D) than in those with cancer of low grade (Grade 1) and low stage (Stage A), respectively. A significant decrease of plasma urokinase inhibitory activity was observed in blood samples obtained 3 weeks after surgical excision of the tumor as compared with that measured before the operation. The physiologic and clinical significances of these findings are discussed.

Key words: Urokinase inhibitor, fibrinolysis inhibitors, bladder cancer.

Introduction

In many reports on fibrinolysis and coagulation disorders in patients with cancer, special attention has been paid to the production of fibrinolytic and thromboplastic enzymes by tumor tissue and the role of these enzymes in the spread and growth of tumors. In 1971, Kojima and Hisazumi (13) quantitified the plasminogen activator in cancerous bladder tissue by the method of Astrup and Albrechsten (2), and found that its level was significantly higher in more malignant tissues than in less malignant ones. Thereafter, Hisazumi et al. (7), using Todd's histochemical method with the necessary modifications, demonstrated that bladder tumor cells had no fibrinolytic activity in either frozen sections or smear preparations of samples from the surface of the tumor. In addition, they showed that intense fibrinolytic activity caused by a plasminogen activator was present in injured or degraded tumor cells. Recently Hisazumi (8) proved inhibitory activity against urokinase and tissue activator in fragments of bladder tumor tissue and their saline extract, the activity was stronger and more selective against urokinase. These studies of local fibrinolysis in tumor tissue suggest that fibrinolysis inhibitors and activators might co-exist in cells with the former supressing the latter, furthermore, diffusion of inhibitors after cell degradation might result in the appearance of fibrinolytic activity in the degraded region. On the other hand, the inhibitors might enter the blood stream and act as an antiplasmin in the blood fibrinolytic system. Furthermore, Hisazumi and Fukushima (9) demonstrated in an experimentally induced rat bladder tumor that plasminogen activator activity gradually decreased as the neoplastic processes in the bladder epithelium were established, and that the ratio between plasminogen activator and urokinase inhibitor was much higher in cancerous tissue (1 : 434) than in normal tissue (1 : 32). Coincidentally, a carcinoma developed following a highly significant decrease in blood fibrinolytic activity.

The present study was conducted to clarify the clinical meaning of urokinase inhibitory activity in both the blood and cancerous tissue of patients with bladder cancer.

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Materials and Methods

Fibrin plate: Bovine plasminogen-rich fibrinogen (Armour Pharmaceutical Co., Kankakee, Ill.) was dissolved in borate-saline buffer (pH 7.75) at a concentration of 0.2%. Bovine thrombin (Thrombin Topical, Parke Davis and Co., Detroit, Mich.) was dissolved in 0.9% saline solution at a concentration of 50 NIH units per ml. Fibrin plates were prepared as described in the previous paper (6).

Urokinase: Urokinase (Green Cross Corp., Osaka) was dissolved in borate-saline buffer. A stock solution containing 500 Ploug units of urokinase per ml was prepared and stored at minus 20° C.

Plasma urokinase inhibitory activity: Citrated venous blood sample (0.5 ml of 3.8% sodium citrate per 4.5 ml of blood) were drawn, and immediately chilled in a cold room, followed by plasma separation. The plasma was serially diluted with borate-saline buffer up to dilution of 8-fold, and to 0.2 ml of these diluted specimens 0.2 ml of a standard urokinase solution (30 Ploug units per ml) was added. The fibrinolytic activity of these mixtures was estimated by the fibrin plate method of Brakman and Astrup (5), and the inhibitory activity was calculated by the method of Kawano et al. (12). One inhibitory unit was defined as the activity necessary to inhibit one Ploug unit of urokinase. This assay system measured the combined inhibition of urokinase and plasmin. For the assessment of urokinase inhibition, therefore, the content of antiplasmin in plasma was simultaneously measured. Plasma was mixed with an equal volume of a standard plasmin solution (25 casein units per ml, Green Cross Corp., Osaka) and a drop (30 µl) of the mixture was applied on heated fibrin plates, which are free in plasminogen (6). Then, the plates were incubated at 37° C for 18 hrs. The plasmin inhibitory activity of the samples was expressed as a percentage of the fibrinolytic activity of the standard solution mixed with an equal volume of borate-saline buffer instead of plasma.

Urokinase inhibitory activity in tissue. Normal and cancerous bladder tissues were rinsed 3 times in 0.9% saline solution to remove coagulated blood, and tissue extracts were prepared by the method described in the previous paper (7). The inhibitory activity was determined by the method of Kawano et al. (12) and expressed in inhibitory units per 1 gm of fresh tissue.

Results

Plasma urokinase inhibitory activity. The plasma urokinase inhibitory activities of 33 patients with bladder cancer were determined and compared to those of the 24 apparently normal individuals used as the control. Tumors were histologically classified by the grading of Broders for malignancy and staging of Marshall for infiltration. As shown in Table 1, all tumors were transitional cell carcinomas and 18 of 33 were Stage A and Grade 1 carcinomas indicating superficial infiltration and low malignancy. Plasma urokinase inhibitory activities of the cancer patients and the normal controls are shown in Fig. 1. The geometric mean (G. M.) of the inhibitory activity level for the control group was 10.7 units per ml with a range of 3.9 to 23.4, while that for the cancer group was 17.5 units per ml with a range of 3.0 to 55.6. There was a statistically significant increase in the latter ($0.01 < p < 0.05$). The highest value was noted in a patient with widespread metastatic carcinoma of the lung. No significant differences in antiplasmin content were observed between the patient and control groups.

Table 1. Pathological classification of 33 cases used for determination of plasma urokinase inhibitory activity with reference to the degree of infiltration and the grade of malignancy

	Grade 1	2	3	4	total
Stage					
A	18	2	0	0	20
B	0	6	1	0	7
C	0	0	4	0	4
D	0	1	1	0	2
total	18	9	6	0	33

The carcinomata were divided into a Stage A group and a Stage B, C and D group, and into a Grade 1 group and a Grade 2, 3 and 4 group. Statistical analysis of the plasma urokinase inhibitory activities grouped was performed. As shown in Figs. 2 and 3, there was a significant increase of inhibitory activity in the high stage (Stage B, C and D group; G. M.: 30.2 urokinase inhibitory units per ml) and high grade (Grade 2, 3 and 4 group; G. M.: 30.0 urokinase inhibitory units per ml) groups compared to the low stage (Stage A group; G. M.: 13.2 urokinase inhibitory units per ml) and low grade (Grade 1 group; G. M.: 12.3 urokinase inhibitory units per ml) groups, respectively ($0.01 < p < 0.05$, $p < 0.01$). No significant differences in antiplasmin content were observed between the samples grouped by the staging or grading.

The effect of surgical resection of tumors upon plasma urokinase inhibitory activity was investigated in 13 patients without direct local invasion, lymphatic permeation, remote metastases and even any of findings suspective for the progressive development of the tumor. The samples were obtained before the operation and 3 weeks after the removal of the tumor, respectively. As shown in Fig. 4, a significant decrease of inhibitory activity

was observed after the operation ($p < 0.01$), although there was a slight increase in 3 samples. The reason for this increase is not clear.

Urokinase inhibitory activity in tissue. Only 10 of the 33 patients, who were subjected to the determination of plasma urokinase inhibitory activity, provided a sufficient tissue volume and quality for this experiment. In addition, 15 frozen fresh cancerous tissues suitable for this experiment, supplied through the courtesy of some sister Hospitals in the Hokuriku area, were used to this determination. In all, these 25 cancerous tissues were histologically classified as shown in Table 2. Fresh normal bladder tissues from 15 autopsy cases, which were kept in a freezing chamber as soon as possible in order to prevent postmortem changes by the courtesy of the Pathological Department of Kanazawa University, School of Medicine, were served as control. None of the extracts from these normal or cancerous tissues showed plasmin inhibitory activity on heated fibrin plates, which agreed with the results obtained in the previous study (8).

As shown in Fig. 5, a significant increase of inhibitory activity was noted in the more infiltrating cancers of the Stage B, C and D group (G. M.: 1159.1 urokinase inhibitory units per gram of fresh tissue with a range of 187.5 to 8100.8) as compared with the less infiltrating cancers of the Stage A group (G. M.: 67.8 urokinase inhibitory units per gram of fresh tissue with a range of 13.6 to 2529.0) ($p < 0.01$). In addition, both groups showed a highly significant increase of inhibitory activity compared to the normal tissue group; 11 of 15 normal specimens exhibited no inhibitory activity and the remainder exhibited 5.1, 5.2, 6.2 and 11.3 urokinase inhibitory units per gram of fresh tissue, respectively. The comparisons based on histological malignancy gave the data shown in Fig. 6. It can be seen from the figure that the high malignancy group (Grades 2, 3 and 4, G. M.: 431.3) exhibited a significant increase of inhibitory activity compared to the low malignancy group (Grade 1, G. M.: 48.7) ($p < 0.01$).

Relationship of plasma and tissue urokinase inhibitory activities. No significant relationship was found between these activities in 10 patients with bladder cancer.

Discussion

Urokinase inhibitor in the blood has been clinically studied by Brakman and Astrup (5) and

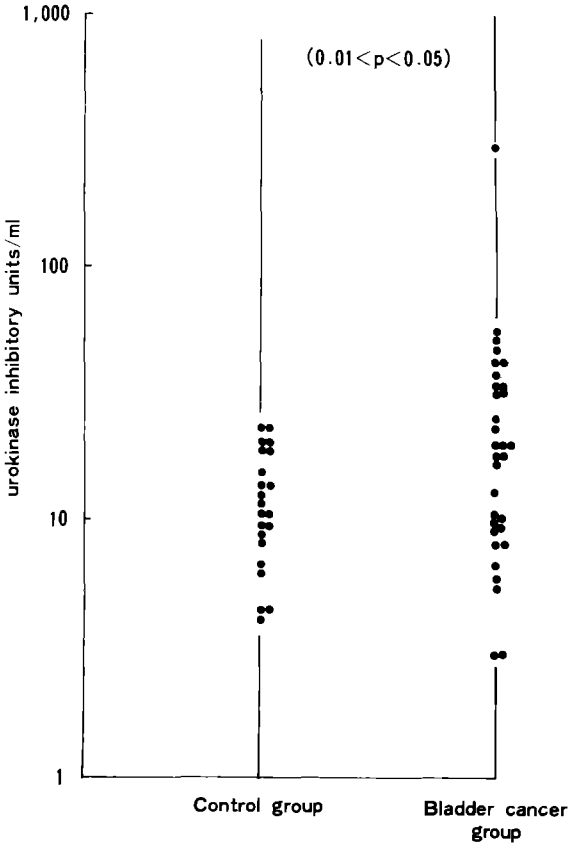

Fig. 1. Plasma urokinase inhibitory activity in control and bladder cancer groups

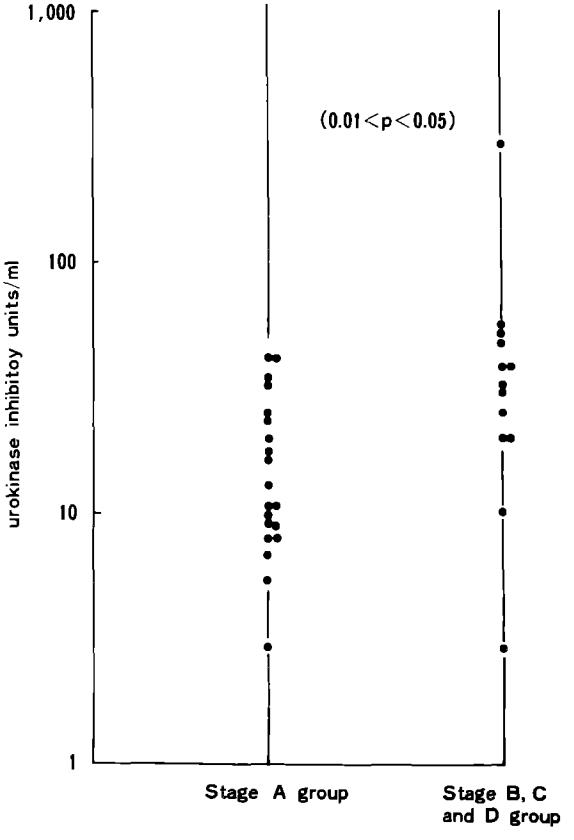

Fig. 2. Plasma urokinase inhibitory activity and degree of infiltration

Lauristen (14). They demonstrated that a significant increase of selective inhibition of urokinase-induced fibrinolysis appeared in the plasma and sera of pregnant women in the 2nd and 3rd trimester. Markedly increased concentrations of urokinase inhibitor in the placenta were confirmed by Januszko et al. (11), Lauristen (14) and Kawano et al. (12). In addition, Uszyński and Uszyński-Folejewska (17) determined plasminogen activator and urokinase inhibitor in myometrium, placenta and amniotic fluid with the result that the ratio of activator to inhibitor in all of these tissues taken together was 1 : 13.9. In addition the plasminogen activator level was highest in the myometrium and the urokinase inhibitor level was highest in the placenta. Åstedt (1) reported that the depression of fibrinolytic activity during pregnancy was due to the presence of the placenta. In cultures of kidney, renal vessel, ureter, bladder, lung and heart from both adults and fetuses, Bernik and Kwaan (3, 4) found that the cells produced an activator antigenically identical to urokinase and others immunologically different from it and that inhibitory activity specific to urokinase was also derived from these culture cells. These findings indicate that inhibitor as well as urokinase may be produced by cells of various organs and may contribute to the regulation of the fibrinolytic system together with antiplasmin.

Table 2. Pathological classification of 25 cases used for determination of cancerous tissue urokinase inhibitory activity with reference to the degree of infiltration and the grade of malignancy

	Grade 1	2	3	4	total
Stage					
A	11	3	0	0	14
B	0	3	3	0	6
C	0	0	3	1	4
D	0	0	1	0	1
total	11	6	7	1	25

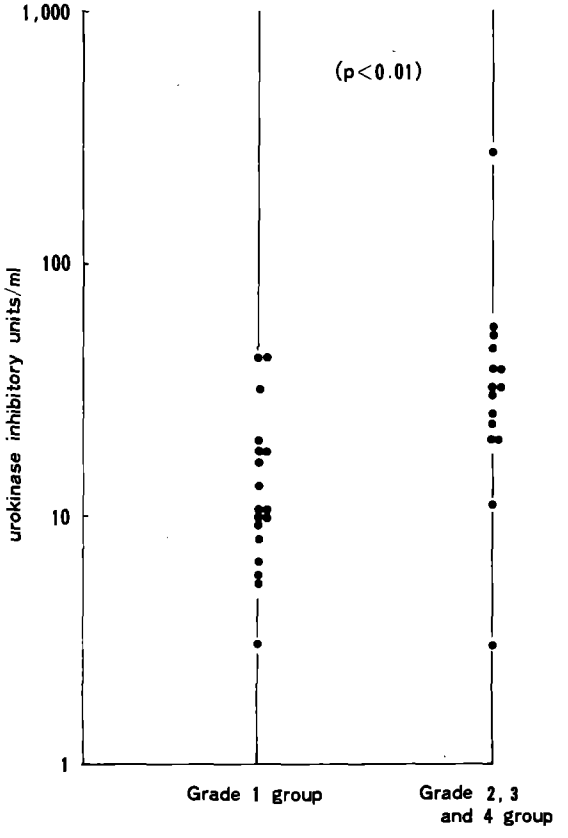

Fig. 3. Plasma urokinase inhibitory activity and grade of malignancy

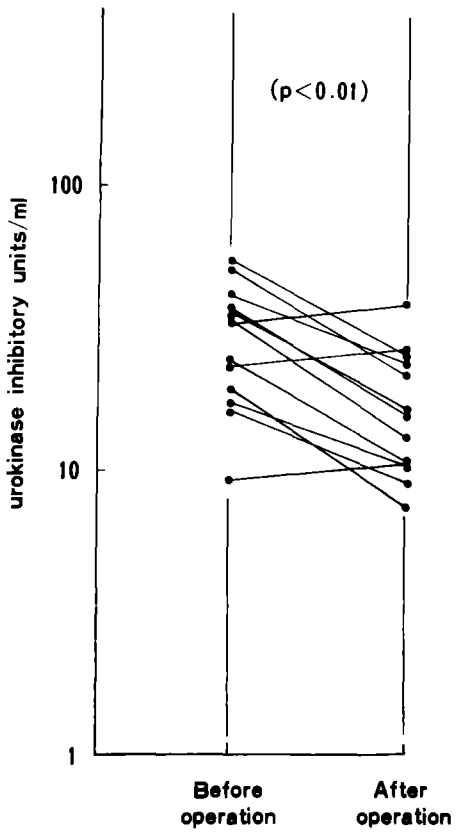

Fig. 4. Effect of surgical extirpation of a tumor on plasma urokinase inhibitory activity

Patients with cancer and especially those with an advanced tumor are prone to develop vascular thrombosis. "Defibrination syndrome", a peculiar form of intravascular coagulation, leads to a generalized hemorrhagic tendency associated with increased fibrinolytic activity in blood in a paradoxical way. Miller et al. (15) demonstrated a "hypercoagulable state" which predisposed patients to excessive bleeding following surgery by shorter silicone coagulation time in a series of 50 patients with cancer. Subsequently, Soong and Miller (16) noted a significantly higher level of urokinase inhibitor and a marked elevation of the plasma fibrinogen level in a group of 100 unselected patients with disseminated malignancies as compared with normal subjects. They reported that antiplasmin and streptokinase inhibitor levels were normal in them. We have very little knowledge about fibrinolysis and its inhibitors in bladder cancer patients. Most urological reports on the role of the fibrinolytic system in malignant neoplasms have been concerned with the unusual cases of severe hemorrhages in prostatic cancer patients with disseminated metastases. The previous study (10) revealed a significant elevation of plasma fibrinogen level and depression of fibrinolytic activity, as measured by euglobulin lysis times, in the group of patients with an advanced bladder cancer. The present study shows that in patients with an advanced bladder cancer there is a selective increase in

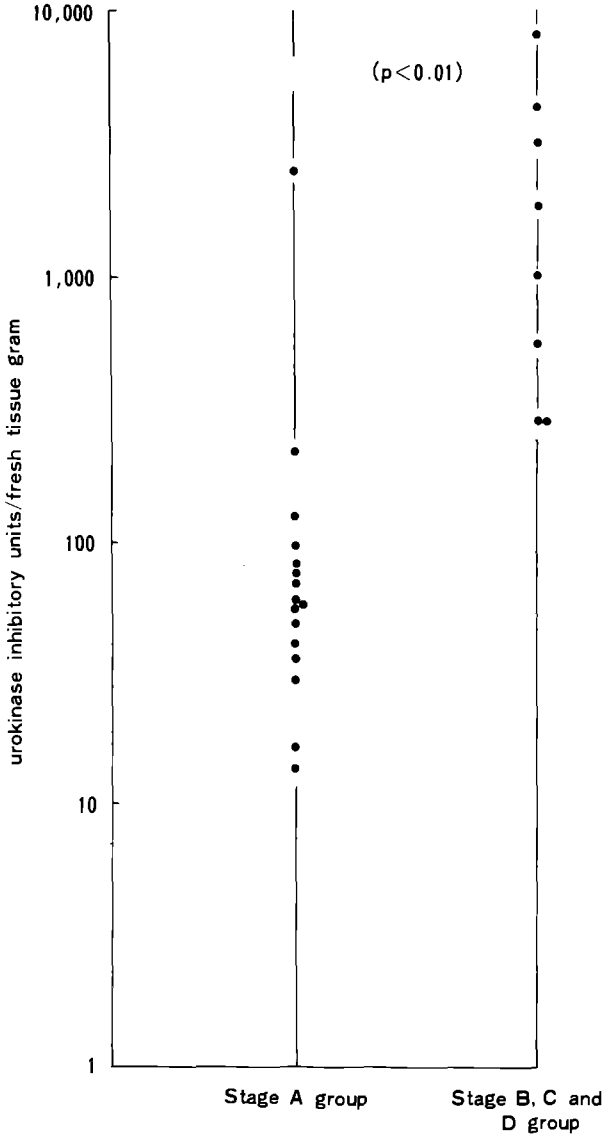

Fig. 5. Urokinase inhibitory activity of cancerous tissue and degree of infiltration

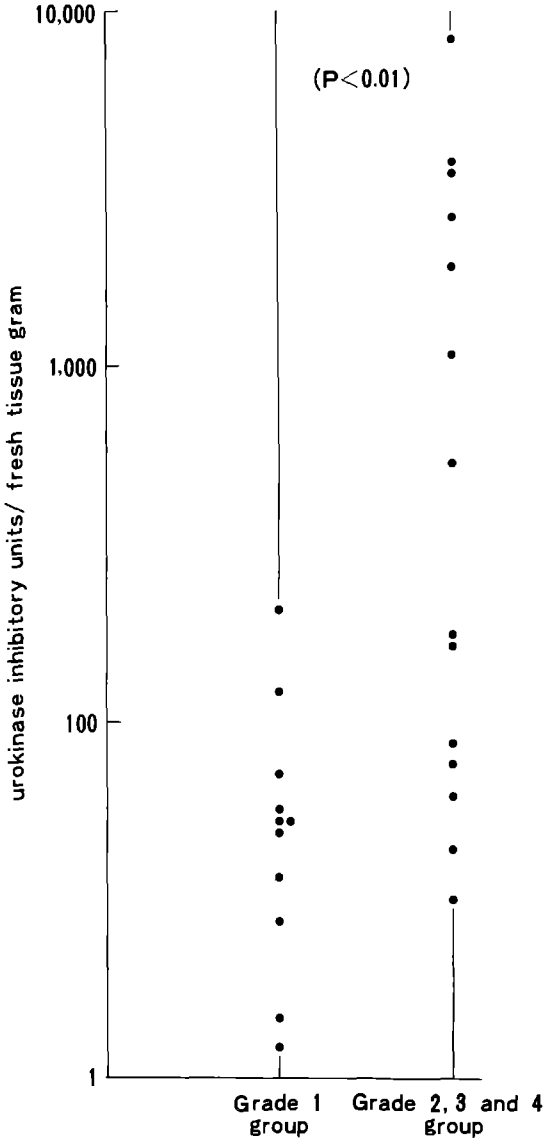

Fig. 6. Correlation between cancerous tissue and plasma urokinase inhibitory activities

the capacity of blood to inhibit urokinase or urokinase-induced fibrinolysis since no increased plasmin inhibition in the plasma was encountered. The increase of plasma urokinase inhibitory activity is greatly in favor of antiplasmin in blood and the fibrinolytic system seems to change in patients with bladder cancer in the direction of depressed fibrinolysis. It is unknown whether the plasma inhibitor against urokinase bears any relationship to the tissue urokinase inhibitor found in increased amounts. It is interesting, however, to note that the increased plasma urokinase inhibitory activity in the patients significantly decreased after removal of the tumor. Based on studies of antiplasmin and antitrypsin in cancer patients and experimental animals, Worowski and Farbiszewski (18) speculated that the enhanced release of the proteolytic enzyme inhibitors into the circulating blood resulted from the increased permeability of the neoplastic cell membranes as a result of necrosis, inflammatory foci and disturbances in the circulation. We observed no linear relationship between urokinase inhibitory activities in plasma and tissue, but this finding doesn't conflict with their speculation. The present investigation as well as our studies (9) on experimental rat bladder tumors induced by the administration of N-butyl-N-butanol (4)-nitrosamine suggests that the tumor tissue participates in the inhibition or activation of local and general fibrinolytic phenomena in tumor bearing patients.

Our results also show that the increase in urokinase inhibition may indicate to some extent the degree of malignancy of bladder tumors, but the mechanism involved in this phenomenon remains still to be elucidated.

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Dr. H. Hisazumi
Dept. of Urology
School of Medicine
Kanazawa University
Kanazawa-City
Japan

Responsible for the text: Prof. Dr. F. Truss, Urolog. Klinik und Poliklinik für Urologie der Univ. Göttingen, Gosslerstr. 10, D-3400 Göttingen.
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