

SERUM 3-HYDROXYANTHRANILIC ACID ESTIMATION  
FOR THE IMMUNODIAGNOSIS OF BLADDER TUMORS

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3-Hydroxyanthranilic acid (3-HAA) was determined by an immunologic method in the blood serum of 115 patients with various bladder tumors, five patients with hemorrhagic cystitis, 47 clinically healthy donors, and 44 patients with acute cystitis. The 3-HAA hapten was found in the blood serum of all patients with hemorrhagic cystitis, 109 of the patients with bladder tumors, and eight of the 44 patients with acute cystitis. It was not found in the donors' serum. The significance of the results for the immunodiagnosis of bladder tumors is discussed.

KEY WORDS: tumors of the urinary bladder; 3-hydroxyanthranilic acid.

An urgent task in oncology is to seek new methods for the diagnosis of malignant diseases. Immunologic methods are promising in this respect because of their high sensitivity and specificity.

The compound 3-hydroxyanthranilic acid (3-HAA) is a carcinogenic metabolite of tryptophan [1, 7]. In carcinoma of the urinary bladder, as a result of a disturbance of tryptophan metabolism in the patients the excretion of 3-HAA in the urine is considerably increased [1, 7]. The writers first suggested and developed an immunologic method of 3-HAA assay in the blood serum of these patients. It was shown experimentally that 3-HAA is readily determined by this method in the serum of animals artificially loaded with 3-HAA and also in the early stages of experimental chemical carcinogenesis [2, 3].

Preliminary results of 3-HAA assay in the serum of patients with bladder carcinoma showed that it is detected by this method in almost 100% of cases [4]. These findings suggested that the determination of 3-HAA in the patients' serum can be used as a diagnostic test for bladder carcinoma in man [5].

This paper describes an investigation of the 3-HAA content by an immunologic method in the serum of patients with bladder tumors in various stages of the disease, and also as a control in the serum of patients with noneoplastic diseases of this organ.

## EXPERIMENTAL METHOD

The serum 3-HAA level was determined by the double diffusion method in agar using immune sera containing antibodies against 3-HAA. The immune sera were obtained by immunizing rabbits with 3-HAA-azoproteins [6]. To obtain the azoproteins the 3-HAA was diazotized in HCl solution and the resulting diazo compound of 3-HAA was azo-coupled with horse serum in an alkaline medium. Rabbits were immunized intraperitoneally in a dose of 0.1 g azoprotein protein per injection daily for 5 days. The course of immunization was repeated after an interval of 3 days. This cycle of immunization was repeated after 2 months. Blood was taken from the marginal vein of the rabbit's ear on the 9th day after the last injection. The resulting immune sera were concentrated twofold, preserved with boric acid, and used for the reactions.

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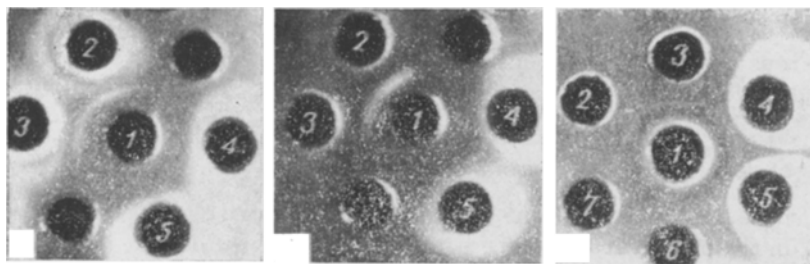


Fig. 1

Fig. 2

Fig. 3

Fig. 1. 3-HAA in serum of a patient with an infiltrating tumor of the bladder in stage  $T_2$ : 1) serum of rabbit No. 213 containing antibodies against 3-HAA; 2, 3) patient's blood serum; 4, 5) donor's blood serum.

Fig. 2. 3-HAA in serum of patient with malignant papillary bladder tumor in stage  $T_2$ : 1) serum of rabbit No. 212 containing antibodies against 3-HAA; 2, 3) patient's blood serum; 4, 5) donor's blood serum.

Fig. 3. 3-HAA in serum of two patients with bladder tumors (identity test): 1) immune serum of rabbit No. 225 against 3-HAA-azoprotein; 2, 3) serum of patient with infiltrating bladder tumor in stage  $T_4$ ; 4, 5) serum of patient with papillary bladder tumor in stage  $T_1$ ; 6, 7) donor's serum.

Blood serum from patients with bladder tumors was investigated. The sera of clinically healthy donors, taken at the First Leningrad Blood Transfusion Station, and the sera from patients with acute cystitis were used as the controls. The rabbit immune sera were exhausted with the serum of healthy donors of the corresponding blood groups. To confirm the identity of the hapten found in the patients' blood serum with 3-HAA, the method of inhibition of the 3-HAA precipitation reaction was used. For this purpose the rabbit immune serum was treated with 3-HAA dissolved in borate buffer, pH 7.8, at the rate of 60 mg 3-HAA to 1 ml serum, after which the serum was tested in the precipitation reaction with those patients' sera that had reacted positively with it before the addition of the 3-HAA.

#### EXPERIMENTAL RESULTS

Altogether 129 patients were investigated, five with hemorrhagic cystitis, 21 with infiltrating bladder tumors, and 94 with papillary tumors. Depending on the stage of the disease, the patients with infiltrating bladder tumors were distributed as follows: one patient in stage  $T_2$ , 12 in  $T_3$ , and eight in  $T_4$ . Of the patients with papillary bladder tumors 36 were in stage  $T_1$ , 43 in stage  $T_2$ , 13 in  $T_3$ , and two in  $T_4$ . The control group consisted of 47 clinically healthy donors and 44 patients with acute cystitis.

3-HAA was discovered by the agar diffusion method in the blood serum of 114 of the 120 patients studied. Evidence of the presence of 3-HAA was given by the formation of a clear precipitation line during the reaction between rabbit immune serum against 3-HAA and the patients' blood serum (Figs. 1-3). No such precipitation line was observed as a result of the reaction of the same rabbit immune serum with the sera of the healthy donors. The specificity of the 3-HAA found in the patients' serum was confirmed by inhibition of this reaction by the 3-HAA hapten.

3-HAA was found in the blood serum of all patients with hemorrhagic cystitis (five) and with infiltrating bladder tumors (21). It was found in the blood serum of 88 of 94 patients with papillary bladder tumors: in 33 (of 36) in stage  $T_1$ , in 41 (of 43) in stage  $T_2$ , in 12 (of 13) in  $T_3$ , and in two patients in stage  $T_4$ .

Of the six patients in whose blood serum no 3-HAA was found, the duration of the disease was very short in four (it began in 1971-1972), and only in two patients with stage  $T_1$  did the disease begin between 1960 and 1970. In addition, these patients had no recurrence of the tumor or not more than one or two recurrences during 5-10 years of the disease. No 3-HAA was found in the serum of any of the 47 healthy donors. 3-HAA was found in the blood serum of eight of the 44 patients with acute cystitis. Considering the earlier observations of the appearance of 3-HAA in the blood serum of animals in the early stages of

experimental chemical carcinogenesis, before the appearance of a macroscopically visible tumor [3], the presence of precancerous changes in the mucous membrane of the bladder can be postulated in patients giving a positive reaction for 3-HAA, before any evidence of them can be revealed by cystoscopy.

Further investigations are required to solve the problem of whether this method can be used for the differential diagnosis of neoplastic diseases of other organs.

In conjunction with earlier observations [4], the results described above thus show that the immunologic assay of 3-HAA in the blood serum can be used as a test for the diagnosis of neoplastic and, probably, preneoplastic diseases of the urinary bladder. It is a very interesting fact that this test proved positive in all patients with hemorrhagic cystitis, characterized by hyperplasia of the epithelium; this confirms that the proposed test can be used to detect precancerous diseases of the bladder. The proposed method of immunodiagnosis could prove extremely useful especially if the diagnosis of bladder tumors is complicated by the presence of marked inflammatory changes in the urinary tract or of strictures of the urethra.

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