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ARTICLE

Urinary Excretion of Thioamine Compounds in Patients with Malignant Tumors and Inflammation*

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The Kokonov's reaction measures thioamine-like compounds excreted in the urine. We compared the results obtained by this reaction in patients with malignant tumors and inflammatory diseases. The majority of patients with advanced (stage III-IV) cancer displayed a positive test. The chance of obtaining a positive test was related to the tumor mass. Lymphoma patients in complete remission were negative. Patients suffering from bacterial infections, active autoimmune diseases, and acute pancreatitis or myocardial infarction also had positive tests. The increased excretion of thioamines, found in tumor-bearing patients can be explained by the activation of the immune system, probably by the inflammation around or within the tumor tissue. The Kokonov's reaction is not suitable to discriminate between tumor-bearing patients and healthy ones. It rather seems to be appropriate to assess inflammatory processes in infections and/or autoimmune diseases. (Pathology Oncology Research Vol 2, No3, 167–170, 1996)

Key words: urinary thioamine, tumor, inflammation

Introduction

More than three decades ago, Kokonov published a paper (in Russian) describing how patients with stomach and other neoplasms excrete large amounts of thioamine compounds in their urine while patients without malignancy only rarely do so. He developed a simple test to detect these compounds.[2] Since his discovery seemed to be suitable for screening, a large cohort of patients has been studied,[3,6] and within this group a much higher proportion of test-positive patients developed malignant tumors, over the same period of observation, than test-negative patients.[1] Therefore, this simple method has been advised for the screening of patients with a high risk of malignancy.[1] By testing the reliability of this reaction in a pilot study, we have found that, indeed, the majority of tumor-bearing patients had a positive Kokonov's reaction, but patients with viral disease, e.g. children with chicken pox also displayed a positive test. This finding prompted studies resulting in the theory that the activation of the immune system was responsible for the increased urinary excretion of thioamines.[5] According to this theory, we analysed randomly selected patients with malignancy and inflammatory diseases, in order to compare their thioamine excretion. In this paper, we report on the results found in tumor-bearing patients and in patients suffering from various forms of inflammation and/or tissue necrosis.

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Abbreviations: CML: chronic myeloid leukemia; HD: Hodgkin's disease; MCTD: mixed connective tissue disease; MM: multiple myeloma; NHL: non-Hodgkin's lymphoma; PM: polymyositis; PSS: progressive systemic sclerosis; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus

Materials and Methods

Detection of thioamines

Urinary thioamines were measured by the method of Kokonov's[2] with modifications. In brief: 100 ml of aqueous solution of 2M selenous acid (SeO_2, Reanal, Hungary) was added to 5 ml of fresh morning urine. Absorbance was measured after 10 min at 560 nm. Positive samples

showed an orange/red/dark red colour. To determine the optimal absorbance, the urine samples of 20 healthy controls and 20 patients with positive results were assayed at 520, 540 and 560 nm. In both groups, the highest absorbance was found at 560 nm *(Fig.1)*, therefore, this wavelength was used for further determinations.

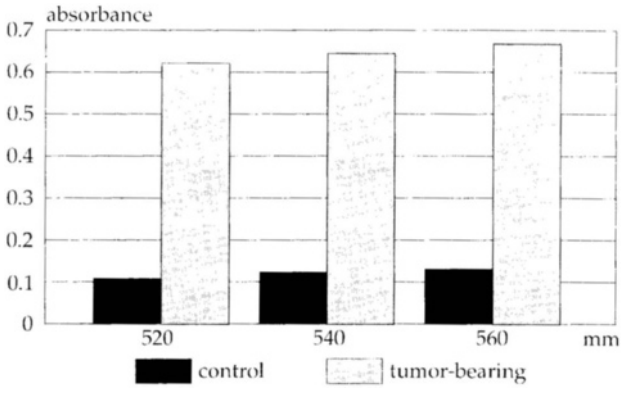

Figure 1. *Absorbance at 520, 540, and 560 nm. Healthy controls and patients with positive reaction (n: 20)*

Patients

The fresh morning urine samples from tumor bearing patients, patients with autoimmune diseases, patients with bacterial infections and other diseases were randomly obtained from the 2nd Department of Medicine. It is of importance that dilution of the urine should be avoided, i.e. diuretics or excess drinking were not allowed. The urine samples of 50 patients with ischaemic heart disease, hypertension, emphysema or peptic ulcer were also studied: These patients were randomly selected as "control patients" and their thorough examination failed to reveal either inflammation or malignancy.

Table 1. **Absorbance values of repeat tests**

Patient Nr.	*Day 1*	*2*	*3*	*4*	*Rating*
a) Healthy volunteers					
1	0.035	0.090	0.00	0.055	(all negative)
2	0.08	0.035	0.045	0.07	(all negative)
3	0.015	0.075	0.055	0.015	(all negative)
4	0.015	0.019	0.085		(all negative)
5	0.0	0.03	0.09		(all negative)
6	0.0	0.0	0.045		(all negative)
b) Tumor patients					
1	0.51	0.54	0.76	0.5	(all positive)
2	0.15	0.79	0.33	0.44	(all positive)
3	0.23	0.3	0.21	0.2	(all positive)
4	0.175	0.25			(all positive)
5	0.3	0.175			(all positive)
6	0.45	0.55			(all positive)

Table 2. **Kokonov's reaction of patients with malignant tumors**

Nr.	*Tumor*	*Stage*	*Remark*	*Rating*
1	colorectal	IV		+
2	colorectal	IV		+
3	colorectal	II		–
4	colorectal	I		–
5	colorectal	III		+
6	colorectal	IV		+
7	colorectal	IV		+
8	colorectal	II		–
9	liver	IV		+
10	liver	IV		–
11	liver	IV		+
12	bronchus	IV		+
13	larynx	IV		+
14	breast	IV		+
15	breast	IV		+
16	breast	II		–
17	ovary	II		–
18	adrenal gland	I		–
19	gall bladder	IV		+
20	kidney	III		–
21	kidney	IV		+
22	kidney	III		+
23	bladder	II		+
24	bladder	II		–
25	bladder	IV		+
26	bladder	III		+
27	bladder	III		+
28	prostate	IV		+
29	HD	IV		–
30	HD	IV		+
31	HD	III		+
32	NHL	III	in remission	–
33	NHL	IV		+
34	NHL	III	in remission	–
35	NHL	IV	in remission	–
36	NHL	III		+
37	NHL	II	in remission	–
38	NHL	III		+
39	NHL	III		+
40	NHL	IV		+
41	NHL	III	in remission	–
42	NHL	III	in remission	–
43	NHL	II		+
44	NHL (MM)			–
45	NHL (MM)			+
46	NHL (MM)			+
47	CML		blastic crisis	+
48	CML			+

Controls

Thirty six healthy young volunteers (age 20-26 yrs, both sexes) were used as controls.

Results

Normal values and range

Urine samples from 36 healthy volunteers were assayed. The mean absorbance value (0.072) + 2SD (0.067) = *0.139* was used arbitrarily as the upper limit of normal values.

Table 3. **The effect of cytostatic therapy on Kokonov's reaction in patients with HD or NHL** (absorbance before and after one week of therapy)

Patient Nr.	*Tumor*	*Stage*	*Treatment*	*Before*	*After*
30	HD	IV	ABVD	0.79	0.3
31	HD	III	COPP	0.25	0.11
33	NHL	IV	CHOP	0.44	0.25
36	NHL	III	CHOP	0.305	0.175
38	NHL	III	BACOP	0.167	0.065
39	NHL	III	BACOP	0.65	0.12

Variability of the results

The absorbance values of repeat tests are shown in *Table 1*. While there was a great variation among absorbance values determined after two to four consecutive days, the values remained within the negative range in the control group and within the positive range in the tumor group.

The results of tumor-bearing patients are shown in *Table 2*. The overall frequency of positive cases in solid tumors was 19/28. There was a much higher frequency of positive test among patients with advanced and metastatic cancer (stage III-IV solid tumors: 18/20 vs. stage I-II solid tumors: 1/8. The overall frequency of positivity in lymphomas and leukaemias was 12/20. Of 12 NHL patients, 6 were in complete remission, and none of them were positive, while all patients with involved lymphatic tissue, before therapy, were positive. The effect of cytostatic therapy is shown in *Table 3*. There was a considerable decrease in absorbance after one week of combined therapy, even though results did not return to normal range.

The results of other – inflammatory – diseases are shown in *Table 4*. Fifteen of 29 autoimmune patients were positive. Positivity seemed to be associated with disease activity: in the active SLE group the frequency of positive test was 9/11, while only 1/9 was in the inactive group. All patients with bacterial infection were positive (6/6), similarly, all patients with tissue necrosis, i.e. acute pancreatitis or myocardial infarction were positive (9/9). Eight of 50 patients suffering from diseases other than inflammation or malignancy displayed a positive test (data not detailed).

Discussion

While there was a fairly high proportion of positive test among patients with large tumour burdens, a positive test was less frequent in patients without metastases. In this respect, there was no difference between patients with solid tumours or lymphoma. This is in accordance with Kokonov's findings, who found that complete removal of the tumour resulted in a negative test, while palliative operation failed to alter positive tests.[2] It is also of interest that all patients with NHL in complete remission were negative, while all NHL patients with a documented tumor mass were positive. Cytostatic therapy considerably reduced thioamine excretion after one week, well before measurable regression of lymph nodes were seen. Kokonov also observed that irradiation of patients abolished positive tests; he explained this phenomenon by the effect of the irradiation ensued free radicals.[2] Free radicals may oxidize thiol compounds, therefore, they cannot be detected by this reaction. This possibility cannot be ruled out, since cytostatic drugs, like cyclophosphamide, do produce free radicals. In our opinion, however, the dimin-

Table 4. **Kokonov's reaction of patients with autoimmune and inflammatory diseases**

	Disease	*Activity*	*Rating*
49	SLE	active	–
50	SLE	active	+
51	SLE	active	–
52	SLE	active	+
53	SLE	active	+
54	SLE	active	+
55	SLE	active	+
56	SLE	active	+
57	SLE	active	+
58	SLE	active	+
59	SLE	active	+
60	SLE	inactive	–
61	SLE	inactive	–
62	SLE	inactive	–
63	SLE	inactive	–
64	SLE	inactive	–
65	SLE	inactive	–
66	SLE	inactive	+
67	SLE	inactive	–
68	SLE	inactive	–
69	MCTD	inactive	–
70	MCTD	active	+
71	MCTD	active	+
72	PSS/PM	active	–
73	PSS	active	+
74	PSS	inactive	–
75	PM	active	+
76	RA	inactive	–
77	vasculitis	active	+
78	sepsis		+
79	cholangitis		+
80	cholecystitis		+
81	bronchitis		+
82	bronchopneumonia		+
83	sepsis		+
84	acute pancreatitis		+
85	acute pancreatitis		+
86	acute pancreatitis		+
87	acute pancreatitis		+
88	acute pancreatitis		+
89	acute pancreatitis		+
90	acute pancreatitis		+
91	myocardial infarction		+
92	myocardial infarction		+

[illegible]

processes cannot be ruled out either.

In former experiments [illegible] with known [illegible] stimulatory activity [illegible] in experimental animals, increased urinary [illegible] excretion. This clearly indicates that not [illegible] destruction, but rather activation of cells of the immune system is responsible for this phenomenon. The positive test, found in the majority of tumor-bearing patients, therefore, can be explained by an activation of the immune system, probably by the inflammation around or within the tumor tissue. This supposition, however, requires further studies.

From this standpoint, of course, "Kokonov's reaction" cannot be recommended for discriminating between tumor-bearing patients and healthy ones. It rather seems to be appropriate for either assessing the tumor mass and/or immune reactivity against tumors. This area merits further investigations. The test also seems to be suitable to assess inflammatory processes in infections and [illegible]

[illegible]

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