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# EXCRETION OF 6-AZAURACIL RIBOSIDE IN CANCER PATIENTS

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**Abstract**—The writers studied excretion and retention of 6-azauracil riboside after a single dose of 1 g in thirteen different types of malignant and three non-tumorous diseases, altogether twenty-one cases. In four cases, the excretion was followed for a prolonged period of time. In about 50 per cent of the cases there was a total, or nearly total, excretion of unmetabolized 6-azauracil riboside. In the remaining 50 per cent, partial retention and metabolization occurred. In several cases also, deribosidation of 6-azauracil riboside was noted. In cases with prolonged 6-azauracil riboside application, no therapeutic effect on the basic malignant process could be observed. The drug was well tolerated by all patients, and not even during prolonged administration were there toxic or other side-effects noted.

IN SOME experimental tumours, 6-azauracil riboside (6-AzUR) proved to be incorporated in tumour tissue in greater quantities and more selectively than 6-azauracil (6-AzU) alone.[1] According to experimental results, its cytostatic effect is about ten to twenty times greater than the effect of 6-AzU.[2, 3] At the same time, 6-AzUR has a lesser affinity for the central nervous system than 6-AzU, which makes it more suitable for clinical application.[1] In clinical application of 6-AzU, there appear, in about one-third of cases, symptoms on the part of the central nervous system, which render its further or prolonged administration impossible.[4]

When clinically testing the efficiency of a new cytostatic compound the usual procedure is that it is administered in such cases where there is no longer a danger that another, more efficient therapy might be missed, i.e. in advanced cases. However, the advanced stage of a process by itself and—as it is usual in such a case—foregoing treatments are not a suitable ground for the testing of a new cytostatic drug, quite apart from the fact that the evaluation requires a comparatively long time.

6-AzUR lends itself readily to chromatographic isolation and determination. We availed ourselves of this opportunity to obtain information about its retention by the human organism. In so doing we started from the idea that, should this cytostatic be effective also in human tumours according to a similar or identical mechanism, as supposed by Škoda and Šorm,[5] it is necessary that:

(1) the balance of the 6-AzUR administered should be negative; and

(2) it should be more negative in patients with malignant disease than in healthy individuals or patients with non-tumorous disease.

## EXPERIMENTAL SECTION

Patients were given a purine-free diet. Three days before administration of 6-AzUR, samples of whole-day urine were chromatographed, according to a method described below, to make sure that the patient did not excrete any substances that might interfere with 6-AzUR determination. On the fourth day, the patient received 1·0 g of 6-AzUR intramuscularly, and we followed its excretion daily until its disappearence, or at least for 3 days.

Chromatographic determination of 6-AzUR was carried out photometrically from the eluates of spots at 258 m$\mu$, calculated[5] from $\epsilon = 6{\cdot}2 \times 10^3$. Chromatography was performed in butanol–water system on Whatman paper no. 4. In the urine in which 6-AzUR determination was carried on, purine bases were precipitated before chromatography and the urine was deprived of salt according to the following method, developed by us:

$$1 \text{ ml urine} + 1 \text{ ml } 10\% \ AgNO_3 + 1 \text{ ml } 0{\cdot}1 \text{ N HCl}$$

In this reaction, chloride ions and purine bases are precipitated, while 6-AzUR remains soluble in the acid solution. Subsequently, the precipitate was filtered and residual $AgNO_3$ in the filtrate was removed by addition of an equal volume of 1 N HCl to the filtrate. After filtration of the AgCl precipitate, 0·2 ml of filtrate was subjected to chromatography.

By the method of photometric determination of 6-AzUR from chromatograms, the following values were obtained:

(1) Determination of 6-AzUR from the aqueous solution, chromatographed directly: spread on the spot: 200 $\mu$g; average of determinations: 185 $\mu$g; $\sigma$ of single determination $= \pm$ 10 $\mu$g (sixty-four determinations).

(2) Determination of 6-AzUR from the aqueous solution, treated before chromatography according to the above mentioned method for urine: spread on the spot: 133·3 $\mu$g; average of determinations: 97 $\mu$g; $\sigma$ of single determination: $\pm$ 13 $\mu$g (thirty-five determinations).

(3) Determination of 6-AzUR added to urine, treated according to the above method: spread on the spot: 66·7 $\mu$g; average of determinations: 55 $\mu$g; $\sigma$ of single determination: $\pm$ 5 $\mu$g (seven determinations).

## RESULTS

Results of a single test as to the retained amount of 6-AzUR in twelve different types of tumorous diseases (altogether eighteen patients) and in three patients with non-tumorous disease are shown in Table 1.

As it appears from Table 1, only in two patients with lymphogranulomatosis was the total amount of 6-AzUR retained for a period of 3 days following administration. Seven patients excreted nearly all of the 6-AzUR administered within 24–72 hr after administration. If we take the fact into consideration that, according to our method of determination, lower values were generally obtained (see the Experimental section), we feel justified in supposing that, in about 50 per cent of the patients under test, 6-AzUR was not retained at all.

In four patients (nos. 4, 12, 13 and 18 in Table 1), 6-AzUR was applied for a longer time, excretion being ascertained daily.

In the first of these four patients (no. 4), 6-AzUR was administered for a period of 22 days, namely the first 7 days 2 g daily, then 9 days 5 g and the remaining 6 days again 2 g daily. In the course of application, spots with $R_f$ in the region of 6-azauracil and with an absorption curve similar to that of 6-AzU in the region 220–300 mμ appeared in chromatograms eight times in irregular intervals. After application of a

TABLE 1. AMOUNT OF 6-AzUR RETAINED (DOSE GIVEN 1 G)

| Diagnosis | Retained amount (g) |
|---|---|
| (1) Lymphogranuloma malignum* | 1·0 |
| (2) Lymphogranuloma malignum† | 0 |
| (3) Lymphogranuloma malignum susp.‡ | 1·0 |
| (4) Carcinoma pulmonum | 0·75 |
| (5) Carcinoma pulmonum | 0·3–0·5 |
| (6) Carcinoma pulmonum | 0 |
| (7) Leukaemia acuta | 0 |
| (8) Leukaemia acuta | 0·83 |
| (9) Leukaemia myeloica chronica | 0·70 |
| (10) Leukaemia lymphatica chronica | 0·45 |
| (11) Neoplasma mediastini | 0 |
| (12) Carcinoma mammae, metast. ad pulmo | 0·1 |
| (13) Neoplasma renis | 0·46 |
| (14) Carcinoma ventriculi | 0·15 |
| (15) Carcinoma cutis | 0·65 |
| (16) Carcinoma cutis | 0·0–0·2 |
| (17) Sarcoma regionis supraclavicularis | 0·8 |
| (18) Melanoma malignum | 0·4 |
| (19) Ulcus ventriculi | 0 |
| (20) Diverticulum oesophagi | 0·24 |
| (21) Panmyelopathia | 0 |

* Histologically proved, within three years two courses of HN3, a short time ago sudden death due to cerebral tumour, no lymphogranuloma could be proved by biopsy.

† Clinically typical, histologically proved lymphogranuloma.

‡ Clinically and histologically suspect lymphogranuloma, now being followed up, without symptoms.

single dose of 1·0 g, this patient retained about 0·75 g during a period of 72 hr. When given a daily dose of 2 g, he retained about 0·6 g daily and, after administration of a daily dose of 5 g, he retained on an average 0·77 g daily. In this case, retention of 6-AzUR was not proportional to the increasing dose of 6-AzUR.

The second patient (no. 12), who, after a single dose, retained 0·1 g, received 2 g of 6-AzUR for a period of 13 days and retained 0·2 g on an average.

The third patient (no. 13), who after the first administration of a single dose retained 0·46 g, was given 2 g daily for a period of 11 days, of which he retained daily 0·8 g on an average.

The fourth patient (no. 18), who, after single application of the drug, retained 0·4 g, was given a daily dose of 2 g for 9 days, retaining on an average 0·6 g per day.

In three of these patients (nos. 4, 12 and 13), Endoxan was administered simultaneously. We did not, however, see any therapeutic effects either at the time immediately after administration of the two drugs or during the later course of the disease.

In none of these four patients, who received 6-AzUR for a prolonged period of time, did we note any toxic or other side-effects during the time of administration

(patient no. 4 was given a total dose of 72 g). The drug was well tolerated by all patients. There was only marked local pain during intramuscular injection; 6-AzUR was therefore administered together with procain.

## DISCUSSION AND CONCLUSIONS

In the above tests of the balance of 6-AzUR administered to individuals, the drug passed unmetabolized out of the body in about 50 per cent of the cases. In the remaining 50 per cent, it was retained to a certain extent, but also in these cases, as far as it was excreted, it remained unmetabolized. Only three cases were strongly suggestive of the fact that it became partially deribozidated, since in the chromatograms there appeared spots with $R_f$ and extinction curve characteristic of 6-azauracil.

There was no difference in the degree of 6-AzUR retention between the patients with malignant disease and the three patients with other diseases. It is perhaps interesting to note that, from three cases of lymphogranuloma under investigation, in one instance the whole amount of 6-AzUR was excreted, while in two instances all of it was retained or became metabolized. We feel inclined to believe, however, that excretion and/or retention of 6-AzUR are subject to fluctuations, since in patients where we studied excretion from day to day for a prolonged period of time, comparatively great fluctuations in the amount excreted were noted, but no instance of total retention.

We endeavoured to include in our series, cases with different types of malignant disease. In doing so we wanted to ascertain differences, if any, in 6-AzUR retention between single types of tumours. The response of single types of tumour to a given cytostatic differs greatly, and is also often different in one and the same type of malignant disease. We were therefore anxious to include in our first investigation a somewhat wider range of tumours. However, with the exception of the remarkable instance of total 6-AzUR retention in two cases of lymphogranuloma, it does not seem to us that we could draw further conclusions from the other types of tumorous disease included in the present series. It is also possible that the method employed by us for the determination of 6-AzUR excretion is not accurate enough to ascertain minuter changes in the excretion which would be necessary to answer the question that we put to ourselves in the present experiment.

In most cases, after repeated doses the amount of 6-AzUR retained by the organism did not exceed a certain limit. This finding might be of significance when one goes deeper into the question of therapeutic utilization of this drug. We believe that, in order to obtain such levels of 6-AzUR in tumour tissue as might be required for the inhibition of metabolic processes of nucleic acids, it is necessary to search for new forms of application (administration at shorter intervals, local application, chemical substitution of 6-AzUR leading to increased retention).

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