TAURINE AS A BIOLOGICAL DOSIMETER. ITS DETERMINATION IN PHYSIOLOGICAL SAMPLES BY REVERSED PHASE HIGH-PERFOMANCE LIQUID CHROMATOGRAPHY.

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ABSTRACT

Taurina, which is the metabolic end-product of cysteine shows a dose dependent change in urinary excretion after radiation exposure. The results of whole body gamma irradiated rats with doses of CO-60 ranging from 100 cGy to 800 cGY are expresed as percent increase of taurine in urine in the first 48 hours postirradiation and confirm the existence of a linear relationship.

INTRODUCTION:

To predict the medical consequences of accidental irradiation,the absorbed dose has to be determined with a sufficient degree of accuracy and precision. It is also essential that the dose information can be produced within an acceptable time period, before clinical symptoms requiring important medical treatment are manifiest. So, known dose effect relationship reproducibility of observations and early availabity of results are some of the basic requirements for practical applicability of bio-indicators.

Biochemical analysis of the metabolic patterns in the body can provide information on cellular disturbances that may arise due to the radiation exposure.

There is a general increase in the levels of aminoacid in the urine of animals and humans during the first day after irradiation.

The relative enhancement depends on the absolute excreted amount and on the metabolism of the specific aminoacids. Accordingly, the excretion of aminoacids is not usually an appropiate indicator.

However, taurine, a metabolic end product of the aminoacid cysteine, shows a dose dependent change in urinary excretion after irradiation.

Raghavan et al. (1) showed a linear dose-response relationship in urinary taurine of rats measured at 24 Hs. postirradiation with dose ranging from 100 cGy to 800 cGy of X rays.

In this work we estimated taurine in urine collected during the first 48 hours postirradiation, and the results are expressed as percent increase respect to control value.

MATERIALS AND METHODS:

Normal male rats (Wistar) weighing 200-250 g, mainteined on stock laboratory diet, were used in the experiments.

Whole body gamma irradiation was carried out with Co-60 Picker C4M60 unit at a rate of 29.3 cGy/min, total doses ranging 100 to 800 cGy.

The rats were housed in individual metabolic cages for urine collection. The urine was collected at intervals of 24s. , in flasks containing a few drops of toluene.

It was ensured that there was no contamination of urine by water and faeces.

Taurine was analysed by reversed-phase HPLC after mixed-bed ion-exchange clean up and precolumn derivatization with Dansyl Chloride based on the method of Marquez et al. (2).

Briefly, 1 ml samples of urine were deproteinized adding 0.1 ml 3.2 M of perchloric acid and filtered through an ion-exchange clean up column to remove aminoacids that could interfere with taurine separation by HPLC. Columns were prepared according to Larsen et al. (3).

Separation of Dansyl derivatives was carried out on a 5 µm Supelcosil LC-18 reversed phase column (150 x 4.6 mm I.D., Supelco, Bellefonte, PA, U.S.A.).

Two mobile phase were used: a) methanol 14%, b) 0.6% acetic acid with 0.008% triethylamine in water solution (30:70). It was delivered at a flow rate of 1.5 ml/min and constant room temperature. Detection at 250 nm of wavelength were performed.

RESULTS:

The excretion values of urinary taurine of rats at 24, 48 and 72 hours after exposure are shown in table 1.

TABLE 1

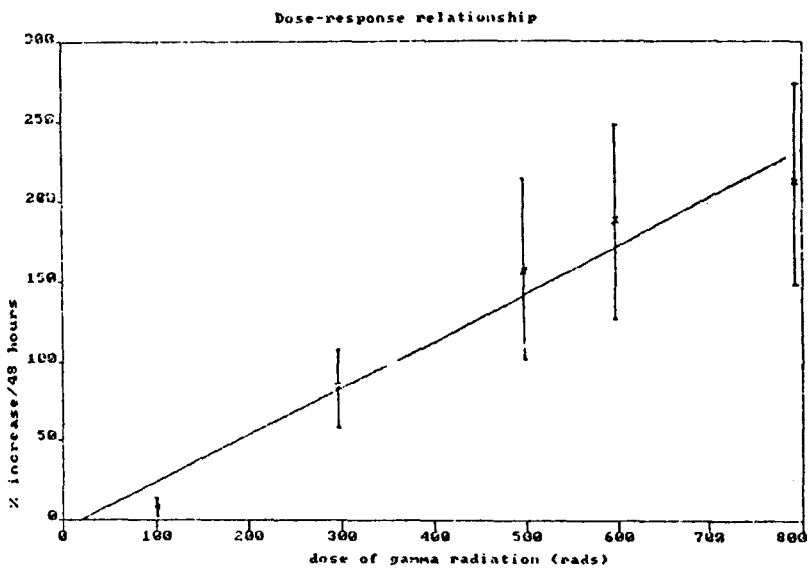
URINARY EXCRETION OF TAURINE (μmoles/24 hours)

Dose (cGY)	100	300	500	600	800
Control*	76.0	87.2	77.1	30.4	50.
S.D.	30.1	35.0	31.6	9.9	19.2
24 Hours	57.8	126.7	130.0	52.0	100.6
S.D.	20.2	53.2	49.0	16.6	27.2
48 Hours	24.2	32.2	67.3	36.5	57.7
S.D.	7.7	10.2	20.1	14.6	17.8
72 Hours	38.7	60.3	47.2	36.1	28.9
S.D.	16.6	20.7	13.2	10.1	6.6

* Before Irradiation

Taurina levels reach a peak at 24 hours and return to normal values at 48 hours. After 72 hours, taurina levels decrease below control values.

The increase during the first 48 hours postirradiation taken in percentage of controls were plotted against doses and a linear correlation was found (fig. 1).



Each point is the average of at least 4 rats.

DISCUSSION:

A number of studies have shown an enhancement in the urinary excretion of taurine after accidental exposures and in irradiated rats and mice.

The present results confirm that increase and the existence of a linear relationship up to a dose of 800 cGy. The lower limit of dose detection is restricted to 100 cGy.

However, the values obtained are not in agreement with the findings of Raghavan and Nadkarmi showing a persistence of high taurine levels after 72 hours of high dose irradiation. We have found a decrease of the mean values in irradiated samples below of control ones, at that time.

It may be questioned the validity of this assay pointing out the relatively great fluctuation in the "normal" physiological taurine status in urine and plasma, but this level is restored after 48-72 hours postirradiation and can be estimated for each individual.

The technique used provides accurate and precise quantitation of taurine in biological materials within 70 minutes.

The level of urinary taurine may be useful for high dose ranges in the early evaluation of radiation injury.

REFERENCES:

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