

Effects of Carbon tetrachloride and Liv.52 on the Clearance Rate of ¹³¹I-Rose Bengal in Rat Liver

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ABSTRACT

¹³¹I-Rose Bengal clearance test has been immensely used for the clinical assessment of functional hepatic abnormalities. It has been observed that external collimated scintillation probe employing ¹³¹I-Rose Bengal is a rapid and less erroneous way of assessing polygonal cell function in liver. The present study demonstrates the protection of liver by Liv.52 from the deleterious effects of carbon tetrachloride (CCl₄) by determining the biological half-life of ¹³¹I-Rose Bengal in male albino rats. An increase in the biological half-life of the radio-iodinated dye was observed following CCl₄ intoxication, which was reversed by Liv.52 treatment.

INTRODUCTION

The liver, because of its strategic anatomical location and its large capacity for metabolic conversions is continuously exposed to different kinds of xenobiotics and therapeutic agents. The rapidly growing morbidity and mortality rates from liver diseases due to drugs and chemicals in industrial nations is largely attributable to the increasing number of noxious medicinal agents and environmental pollutants¹. Till to-date, a large number of experimental hepatotoxic agents have been described, of which carbon tetrachloride (CCl₄) is the most investigated. Carbon tetrachloride inflicts wide-ranging effects on liver metabolism, including adverse reactions on DNA, RNA protein synthesis and necrosis of hepatocytes and cirrhosis^{2,3}. This is supported by the evidence that a few hepatoprotective agents such as Cystamine⁴, Silamyrin⁵ and Malotilate⁶ prevent CCl₄ mediated hepatic injury. Protection against CCl₄ induced hepatic disorders by the multiherbal agent Liv.52 has also been reported by some investigators^{7,8}. Similarly Liv.52 is found to be protective in severe cases of infective hepatitis, chronic active hepatitis and cirrhosis of liver⁹. However, the major problem lies in designing a specific and most versatile technique to analyse the functional status of a particular organ. A number of experimental models have been designed to assess the aetiopathogenesis of liver (both pathologically and clinicobiochemically), amongst which Rose Bengal (tetraiodotetrachlorofluorescein) clearance test has been in widespread use since 1923 owing to its property of being absorbed only in the polygonal cells of the liver^{10,11}. Interest in using Rose Bengal was revived by labeling the dye with ¹³¹I¹². It has been observed that an external collimated scintillation probe provides a quantitative expression for clearance of the dye from blood, and is a fast, simple and relatively less erroneous way of assessing polygonal cell function¹³. The clearance pattern of ¹³¹I-Rose Bengal from liver following CCl₄ and Liv.52 treatment in experimental subjects is unknown. Hence we wished to study the variations in the biological half-life T_(biol) of ¹³¹I-Rose Bengal in liver and the distribution of the dye in different organs in CCl₄ intoxicated and Liv.52 treated rats.

MATERIAL AND METHODS

Mature male albino rats in the weight range of 150-180g were procured from the Central Animal House, Panjab University, Chandigarh. The animals were fed with pelleted standard laboratory feed (supplied by Hindustan Levers Ltd., Bombay) and water ad libitum.

In all, 18 rats were divided into three groups of six each. Group 1 served as control (untreated). In Group 2 and 3, animals were injected subcutaneously (s.c.) with 0.2 ml of CCl₄ mixed with 0.2 ml of groundnut oil twice a week. Moreover everyday the animals in Group 3 were also given orally 0.5 ml of Liv.52 (Himalaya Drug Co., Bombay)⁸. The animals in the Group 1 were injected subcutaneously with only 0.2 ml of groundnut oil twice a week to served as control. All treatments continued for six weeks.

After 6 weeks the animals in all the groups were injected with 0.37 MBq of ¹³¹I-Rose Bengal (procured from Bhabha Atomic Research Centre, Bombay) into the penis vein under light ether anesthesia to determine the biological half-life of the labeled dye in liver and its biodistribution in some organs. Immediately after the injection of the radiopharmaceutical, the anatomical position of the liver was located with the help of a sensitive radiation monitor and was marked. For the measurement of ¹³¹I-Rose Bengal uptake *in vivo*, only this marked area of the liver was exposed through a hole in a specially designed lead shield (Dimensions; length-15.0 cm, width-8.5 cm, thickness-2.0 cm and a central hole of diameter –1.5 cm) which was placed on a NaI (TI) crystal and was counted 10 minutes after the injection and at different time intervals till 70 minutes.

Immediately after the final uptake after 70 minutes, the animals were sacrificed under light ether anesthesia and various organs viz., liver, kidney, heart, spleen and intestine were removed, weighed and digested in equal volumes of 30% KOH for the assessment of retention of activity in them. For the determination of biological half-life of ¹³¹I-Rose Bengal in liver, the maximum uptake, which was observed after 10-15 minutes, was taken as 100%. A standard source was also counted for the possible instrumental errors or variations, if any. Percent uptake values at various time intervals were plotted on a semilog paper. Statistical analyses of the results was done using student's 't' test.

COMPOSITION OF Liv.52

Each 5 ml of Liv.52 syrup contains: *Capparis spinosa* (34 mg), *Cichorium intybus* (34 mg), *Solanum nigrum* (15 mg), *Cassia occidentalis* (8 mg), *Terminalia arjuna* (16 mg), *Achillea millefolium* (8 mg) and *Tamarix gallica* (8 mg).

RESULTS AND DISCUSSION

The results of our present study revealed a significant increase ($p < 0.001$; 31.61%) in $T_{(biol)}$ of ¹³¹I-Rose Bengal in liver after 6 weeks of CCl₄ treatment to rats of Group 2, as compared to their respective controls. This is shown in Table 1. However, the animals of Group 3 treated with Liv.52 along with CCl₄ did not show any significant change in the biological half life of ¹³¹I-Rose Bengal in liver.

Table 1: Biological Half life of ¹³¹I-Rose Bengal following treatment with Liv.52 in CCl₄ intoxicated Rats (mean ± S.D. of 6 determinations in each case)

Group	Biological half-life (minutes)
1 (Control)	53.40 ± 4.44
2 (CCl ₄)	70.28 ± 5.84*
3 (CCl ₄ + Liv.52)	55.00 ± 3.62

* Significance of difference from controls $p < 0.0001$

It is well-established that the rate of clearance of clearance of ^{131}I -Rose Bengal decreases in various liver disorders¹⁰. Similar observations were made of Delprat¹⁰ who has shown that elimination of Rose Bengal from the circulation is profoundly affected by injury to liver caused by chloroform. In a few other cases, where either hepatocellular damage or biliary obstruction were the main discernible features, a lag in clearance of the dye was observed^{14,15}.

In the present investigations, the increased $T_{(\text{biol})}$ of ^{131}I -Rose Bengal in livers of CCl_4 treated animals of Group 2 could be attributed to the slower clearance of the radioiodinated dye from the damaged hepatocytes. The other possibility could be the narrowing or partial blockage of the biliary channels, which somehow impede the flow rate of ^{131}I -Rose Bengal to the intestine. However, the animals simultaneously treated with Liv.52 showed nonsignificant changes in the biological half-life of ^{131}I -Rose Bengal, which suggests a protective action of the drug as has been reported earlier^{7,8}. Also the increased $T_{(\text{biol})}$ of ^{131}I -Rose Bengal in liver of animals treated with CCl_4 gets further substantiated from the results of biodistribution studies, wherein increased accumulation of activities in liver (26.58 ± 5.29 to 35.64 ± 4.56 ; $p < 0.01$) and decreased activities in intestine (50.92 ± 8.17 to 40.16 ± 5.33 ; $p < 0.01$) were observed when compared to their respective controls (Table 2). (From the results, it is once again confirmed that in diseased conditions of liver, more of the activity is retained in the liver and is unable to be pushed out into the intestine via biliary channels owing to damage to the hepatic polygonal cells and also to a probable biliary obstruction. Thus, these results suggest that Liv.52 treatment may help in preventing the functional abnormalities of liver, caused hepatotoxic agents.

Table 2: Percentage of distribution of ^{131}I -Rose Bengal in various organs following treatment with Liv.52 in CCl_4 intoxicated Rats (Mean \pm S.D. of 6 determinations in each case) (Percentage distribution of activity as per 100 mg of tissue)

Group	Kidney	Liver	Heart	Intestine	Spleen
1 (Control)	13.14 ± 2.96	26.58 ± 5.29	3.72 ± 0.65	50.92 ± 8.17	5.63 ± 1.08
2 (CCl_4)	13.19 ± 1.89	$35.64 \pm 4.56^{**}$	4.57 ± 1.75	$40.16 \pm 5.33^*$	6.43 ± 1.65
3 (CCl_4 + Liv.52)	1.54 ± 1.94	28.23 ± 3.50	4.12 ± 0.88	47.17 ± 6.33	6.39 ± 0.98

* Significance of difference from controls $p < 0.01$

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