EFFECT OF WHOLE-BODY IRRADIATION ON MICHAELIS-MENTEN CONSTANTS OF MICROSOMAL ENZYME SYSTEMS OF RAT LIVER

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1. Introduction

The microsomal NADPH-linked electron transport system, containing cytochrome *P*-450, catalyses the oxydative metabolism of a number of drugs and foreign compounds in the liver [1]. The activity of this enzyme system reflects the detoxification capacity of an organism. Whole-body irradiation causes, in the liver of male rats 3–5 days after irradiation, a marked fall in the activity of enzymes acting on several drug models: barbiturates [2]; aminopyrine [3,4]; aminophenazone [5]; biphenyl [6]. It has also been reported that NADPH cytochrome *P*-450 reductase, on which the electron transport chain depends, is somewhat altered [6] and that glucose 6-phosphatase, an enzyme independent of this enzyme system, was also decreased [7]. It was also observed that some of these enzymatic activities return to more or less a normal level at 6–8 days after irradiation [6,7], which is in agreement with the acute radiation syndrome in mammals [8].

We have studied the effect of whole-body irradiation on Michaelis-Menten constants of the NADPH cytochrome *P*-450 reductase (EC1.6.2.3.), the oxidative demethylation of ethylmorphine, and glucose 6-phosphatase (EC3.1.3.9.), at 1, 4 and 7 days after irradiation.

2. Materials and methods

2.1. *Animals*

Male Sprague Dawley rats (Charles River France) 150–200 g, fed on a standard commercial diet (Biscuit RS, Extralabo), were used.

2.2. *Irradiation*

Animals were exposed to 900 R of ^{60}Co radiations at 41.5 R/min dose rate at 100 cm target distance.

2.3. *Preparation of rat liver microsomes*

After decapitation of the rats, livers were removed immediately and homogenized in glass Elvehjem-Potter homogenizers on ice-cold saccharose 25×10^{-2} M. The microsome pellets were harvested by centrifugation at 105 000 × *g* as in [9], then suspended in cold phosphate–EDTA buffer, (pH = 7.5) and adjusted to 10 mg protein/ml, measured as in [10], in the presence of sodium deoxycholate 6×10^{-3} M. Crystalline bovine albumin was used as standard.

2.4. *Enzyme assays*

NADPH cytochrome *P*-450 reductase was measured as in [9], with various concentrations of cytochrome *c*. Ethylmorphine demethylase activity was determined as in [11], the colorimetric method [12] being employed for the estimation of formaldehyde formed. The activity of glucose 6-phosphatase was determined as in [13], the inorganic phosphate released being measured as in [14].

The Michaelis-Menten constants were calculated from Lineweaver-Burk plots, with 6 different concentrations of each substrate.

3. Results and discussion

The enzymatic kinetic constants of the 3 enzyme systems are shown in table 1 for the V_{max} values and table 2 for the K_m values.

Table 1
V_{max} values of 3 microsomal enzyme systems of control rat livers and irradiated rats (900 R)

Animals \ Enzymes		NADPH cytochrome *P*-450 reductase[a]	Oxidative demethylation of ethylmorphine[b]	Glucose 6-phosphatase[c]
C		9.10 ± 0.23	15.9 ± 0.65	51.09 ± 2.24
Ir	D + 1	8.44 ± 0.15	11.3 ± 0.17	54.0 ± 1.60
	D + 4	9.26 ± 0.26	6.63 ± 0.14	49.7 ± 0.48
	D + 7	7.78 ± 0.22	8.47 ± 0.15	40.5 ± 0.59

[a] nM cytochrome *c*/min/mg protein
[b] nM fmol/min/mg protein
[c] nM phosphate/min/mg protein

Means values of 3 determinations ± SE are expressed

Abbreviations: C, controls; Ir, irradiated rats; D, means the day on which irradiation was performed

Irradiation produced a marked decrease in the V_{max} of the oxidative demethylation of ethylmorphine and an increase in K_m, V_{max} reaching a minimum on day 4 after irradiation (about ½ normal value) and K_m reaching a maximum on day 4 (~2-fold normal value). On day 1 after irradiation, both parameters were little affected and on day 7 they returned to values near normal.

For the NADPH cytochrome *P*-450 reductase, only K_m was increased on day 4.

The irradiation did not alter the enzymatic kinetic constant of the glucose 6-phosphatase values whatever the time.

These results, concerning the oxidative demethylation of ethylmorphine, are in accordance with those in [4] with aminopyrine demethylase. Our results also show that the radiation effect on ethylmorphine demethylase is different from that on NADPH cytochrome *P*-450 reductase. Indeed for this enzyme system, the decrease of the activity, described in [6], appears as related with the fall of microsomal protein amount, as shown in table 3. As confirmed [5] that the concentration of cytochrome *P*-450 in the microsomal fraction of rat liver was not altered after irradiation, our results suggest that the affinity of substrates has been changed by irradiation, which is in agreement

Table 2
K_m values of 3 microsomal enzyme systems of control rat liver and irradiated rats (900 R)

Animals \ Enzymes		NADPH cytochrome *P*-450 reductase[a]	Oxidative demethylation of ethylmorphine[b]	Glucose phosphatase[c]
C		6.62 ± 0.30	2.04 ± 0.05	3.07 ± 0.45
Ir	D + 1	7.85 ± 0.23	2.52 ± 0.12	2.76 ± 0.15
	D + 4	8.30 ± 0.37	4.55 ± 0.05	3.44 ± 0.08
	D + 7	6.11 ± 0.28	3.30 ± 0.04	3.30 ± 0.10

[a] 10^{-5} M cytochrome *c*
[b] 10^{-4} M ethylmorphine
[c] 10^{-3} M glucose 6-phosphate

Legends as in table 1

Table 3
Protein contents in liver microsomes of control and irradiated rats

Animals		Protein contents
	C	82.3 ± 4.14
Ir	D + 1	63.9 ± 2.5
	D + 4	47.2 ± 2.17
	D + 7	55.8 ± 4.13

Results are expressed as mg protein/whole liver. Means values of 8 experiments ± SE are expressed. Legends as in table 1

with the observation in [3]. Some conformational alteration of the structure of cytochrome *P*-450 or some change in the membrane or environment of this cytochrome, may have occured.

Concerning the glucose 6-phosphatase, there is no correlation between the decrease of enzyme activity during the acute irradiation syndrome and the kinetics properties of this enzyme system. Since they are not altered, the fall of microsomal protein concentration seems to be responsible for the alteration of the enzymic activity.

In conclusion, whole-body γ-irradiation could inhibit microsomal drug metabolism by 2 processes:

1. The reduction of amount of NADPH cytochrome *P*-450 reductase which reduces the electron transport to cytochrome *P*-450;
2. An alteration of the kinetic properties of the ultimate step of the drug reduction.

The effect of cysteamine on processes 1 and 2 was investigated and will be described elsewhere.

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