0014-4754/95/080790-09$1.50 + 0.20

Immunostimulating lipopeptide, LtriP (RP 56142): comparison of the effect on hepatic cytochrome P 450 modulation and radioprotection in male and female of three mouse strains

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Received 20 April 1994; received after revision 6 December 1994; accepted 20 February 1995

Abstract. The sex-dependent effect of lauroyl-L-Ala-D-γ-Glu-L,L-A_2pmNH_2 (LtriP, RP 56142)** on hepatic microsomal cytochromes P 450 (cyt P 450) was studied in three mouse strains NMRI, C3H/OuJ and C3H/HeJ. In NMRI and C3H/OuJ, strains which are responsive to bacterial lipopolysaccharides (LPS-responsive), regardless of the sex of the mouse, significant decrease in the amount of cyt P 450 was observed after LtriP treatment, with a concomitant reduction in ethoxyresorufin-O-deethylase (cyt P 450 1A-dependent) and 7-ethoxycoumarin-O-deethylase activities. This was not seen in C3H/HeJ (LPS-hyporesponsive) mice. These effects may be related to LtriP-dependent cytokine induction, since neither LtriP nor LPS stimulated interleukin-1 (IL-1) secretion by C3H/HeJ macrophages. 11- and 12-hydroxylations (11- and 12-OH) of lauric acid were compared in C3H/OuJ and C3H/HeJ mice. LtriP depressed the total enzymatic conversion of lauric acid in the two strains without modification of the 11/12-OH ratio for C3H/OuJ or male C3H/HeJ mice. However, in females C3H/HeJ mice this decrease was particularly significant and concerned especially the 12-OH activity (a marker of cyt P450 4A family). Although males of the three strains were more sensitive to irradiation than females, LtriP exerted a sex-independent radioprotection on NMRI and C3H/OuJ mice. Its radioprotective effect was illustrated by the preservation of all the enzymatic activities studied in treated NMRI mice, contrary to irradiated control animals. In contrast, for the C3H/HeJ strain, males were not protected by LtriP treatment and, furthermore, females showed a marked sensitization to irradiation.
The effects in CH3/HeJ strain implicate LtriP in the control of cyt P 450 induction and of sensitivity to irradiation independently of IL-1 induction.
Key words. Lipopeptide; radioprotection; immunostimulation; cytochromes P 450.

The lauroyltetrapeptide lauroyl-L-Ala-γ-D-iso-Glu-LL, DD-2,6-A_2pmNH_2-Gly was the first immunostimulating lipopeptide to be described[1]. This compound was representative of a new family of immunostimulants which exhibit in vitro and in vivo immunopotentiating activities preferentially directed towards cellular immunity, as shown by the non-specific resistance of lipopeptide-treated mice during a systemic infection with the intracellular bacterium *Listeria monocytogenes*[2,3]. These immunostimulants enhance interleukin-1 (IL-1) production by murine macrophages and induce the release of colony-stimulating factors (CSF) in the blood of mice, at the same doses as those which protect these animals against bacterial infections[4]. Experiments performed with synthetic lipopeptides containing either L,L-2,6-diaminopimelic acid (LL-A_2pm) or D,D-2,6-diaminopimelic acid (DD-A_2pm) led to the conclusion that only isomers with LL-A_2pm are biologically active in vivo. Among the intermediates of the active lipotetrapeptides, the lauroyltripeptide (LtriP, lauroyl-L-Ala-γ-D-iso-Glu-L,L-A_2pmNH_2, RP 56142) exhibits similar immunopotentiating activities to the relevant lauroyltetrapeptide (LtetraP LL-A_2pm)[5].
In a previous study, we have shown that one day after treatment of female NMRI mice, LtriP decreases the level of hepatic microsomal cytochromes P 450 (cyt P 450) and CCl_4-induced lipid peroxidation, and protects these animals against the lethal lipid peroxidation process of paracetamol poisoning[6]. Recently, we described the ability of LtriP to increase the resistance of female NMRI mice to the lethal effects of γ-ray irradiation[7].
Stimulation of the immune system is implicated in these activities because the non-immunostimulating analog, the lauroyltetrapeptide containing DD-A_2pm, is inactive (LtetraP LL-A_2pm exhibits activities similar to those of LtriP). IL-1 and tumor necrosis factor α (TNFα) may be mediators of the effects of LtriP since

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** All abbreviations used in this article are listed at the end of the text.

this lipopeptide induces the production of mRNA and the secretion of these immunological factors[7], which in turn exert radioprotection[8].

Several immunomodulators such as bacterial lipopolysaccharide (LPS), Bacillus Calmette Guerin (BCG), *Corynebacterium parvum* and micro-particulate β-1,3-D-glucan[9–11] have been studied as radioprotectors. The mechanism of this activity has been attributed to their ability to enhance hematopoietic and immunological responses such as the release by immune cells of radioprotective cytokines IL-1 and TNFα.

Some metabolites of arachidonic acid (biolipids) such as prostacyclin (PGI_2)[12], leukotriene C_4 (LTC_4)[13], a synthetic analog of prostaglandins, 16,16-dimethyl prostaglandin E_2 ($diPGE_2$)[14] and other phospholipid moieties (platelet activating factor, PAF)[15] are also implicated in immunomodulation and have been shown to be effective radioprotectors. However the mechanism by which the immune system promotes radioprotection remains to be elucidated.

Many immunomodulators can modulate oxidative processes or the generation of activated oxygen species[16]. LPS, BCG, IL-1, γ-interferon and its inducers, modulate the expression of the hepatic cyt P 450[17,18]. Some of these xenobiotic-metabolizing enzymes could interact with radiation-induced radicals of biomolecules and reactive oxygen species and act as antioxidants[19].

In order to determine if some cyt P 450s play a role in the radioprotection due to immunomodulation in mice, we decided to study the modification of some of these murine hepatic microsomal isozymes after treatment by LtriP in the absence or presence of irradiation. The influence of the sex of the mice was also examined. Furthermore, to support the possible involvement of IL-1 expression in the LtriP effects, we compared the response of NMRI mice used in our previous studies, to those of two strains known to differ in the ability of their macrophages to secrete IL-1 after LPS stimulation: C3H/OuJ (LPS-responsive mice, lps^n/lps^n) and C3H/HeJ (LPS hyporesponsive mice, lps^d/lps^d). A spontaneous mutation that occurred within the C3H/HeJ strain rendered it highly refractory to endotoxins[20] and specifically to the lipid A moiety of these molecules[21].

Materials and methods

The lauroyltripeptide (LtriP RP 56142), recombinant human IL-1 and recombinant human TNFα were kindly supplied by Rhône-Poulenc Rorer (Vitry sur Seine, France).

Reagents. Benzyloxyresorufin and reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) were from Boehringer-Mannheim (Germany); ethylenediaminetetraacetic acid (EDTA) was from Fluka (Buchs, Switzerland); cytochrome c, ethoxyresorufin, 7-ethoxycoumarin, pentoxyresorufin and LPS of *Salmonella enteridis* were from Sigma (St. Louis, USA); other reagents were from Prolabo Laboratories (Vitry sur Seine, France) or were of the highest purity commercially available.

Animals. NMRI and C3H/OuJ mice were obtained from Iffa-Credo (St Germain L'Arbresle, France) and were used one week after receipt. The C3H/HeJ strain was kindly provided by URA 171 CNRS.

Treatment of animals. Mice were injected intraperitoneally (i.p.) on 2 consecutive days, with 0.5 mg/kg of lipopetide dissolved in 0.5 ml of saline (apyrogen 0.9% NaCl) or 0.5 ml of saline alone for controls. Mice were identified individually and controls and LtriP-treated animals were housed in the same cages.

Irradiation. Groups of two mice (one control and one treated) were placed into a plexiglass container and exposed to a ^{60}Co γ-ray source, such that they received total body irradiation at various doses. The mean dose rate was 1.4 to 1.8 Gy/min. The number of surviving animals was recorded daily for 30 days. Under our conditions, when the hemopoietic syndrome was fatal mice died before 23 days. Statistical evaluation of survival was determined according to the Logrank test[22].

Preparation of microsomes. The liver of each animal was removed immediately after killing and washed with ice-cold saline solution (0.9% NaCl). Hepatic microsomes were prepared as previously described[6]. Briefly, the liver was homogenized in ice-cold 50 mM Tris-HCl buffer, pH 7.4, containing 0.2 M sucrose and 1 mM EDTA and centrifuged for 20 min at 10,000 g and 4 °C. The supernatant was centrifuged for 1 h at 105,000 g and 4 °C and the microsomal pellet, which was suspended in ice-cold 100 mM sodium pyrophyosphate pH 7.4, was centrifuged under the same conditions. The final pellet was homogenized in ice cold 0.1 M potassium phosphate, pH 7.4, containing 20% glycerol, and stored at 80 °C until the enzyme activity was assayed.

Characterization of cyt P 450 and b_5. *Spectrophotometric determination:* Cyt P 450 and cyt b_5 were characterized and quantified spectrophotometrically[23] and correlated to protein levels determined according to Lowry[24].

Enzymatic determinations: 1) Benzyloxyresorufin-O-dealkylase (B.R.O.D.), ethoxyresorufin-O-deethylase (E.-R.O.D.), pentoxyresorufin-O-dealkylase (P.R.O.D.)[25]: 0.2 nmol of hepatic microsomal cyt P 450 were used in order to ensure linear reaction rates for at least 5 min. Reactions were carried out in 3 ml cuvettes at 37 °C using a Kontron Analytical model SFM25 spectrofluorimeter. The reaction mixture, containing 0.2 nmol cyt P 450, 5 μM substrate (10 μl of 1 mM solution in dimethylsulfoxide, DMSO), 0.1 M phosphate buffer, pH 7.6 EDTA 0.1 mM, was equilibrated for 1 min at 37 °C. The reaction was then started by the addition of 180 μM NADPH. The final reaction volume was 2 ml. The reaction rate was measured directly from the increase in the fluorescence of the resorufin pro-

duced from each of the substrates used. Excitation and emission wavelengths were 530 nm and 585 nm respectively. After a suitable reaction period, the fluorescence increase was calibrated by adding a 10 µl aliquot of authentic resorufin (10 µM in DMSO) to the cuvette.

2) 7-ethoxycoumarin-O-deethylase (7-E.C.)[26]: a standard incubation mixture for the ethoxycoumarin deethylation reaction was prepared by adding 50 µM of substrate (0.1 ml of 1 mM solution in Tris-HCl buffer, pH 7.6, or in H_2O-methanol (9:1; v/v)), 0.5 nmol of hepatic microsomal cyt P 450, and 0.1 M phosphate buffer, pH 7.6. After 1 min at 37 °C, the reaction was started by the addition of 180 µM NADPH. The final reaction volume was 2 ml. The excitation and emission wavelengths were 360 nm and 460 nm, respectively. A known amount of 7-hydroxycoumarin (10 µM) was added at the end of the reaction to calibrate each assay.

3) Lauric acid hydroxylation[27]: ^{14}C lauric acid (100 µM) was incubated in 0.5 ml phosphate buffer containing 1 mg of microsomal protein. The reaction was initiated by the addition of 1 mM NADPH. After a 10 min incubation, the reaction was stopped with 500 µl of methanol containing 1 M HCl. Lauric acid and its metabolites were extracted with ethyl acetate and the extracts were concentrated under vacuum. The pellet was dissolved in buffer A (35% CH_3CN, 0.1 M ammonium acetate, pH 4.6) and separated by HPLC on a 5 µm C18 nucleosil column (125 × 4.6 mm) in buffer A (1 ml/min). The radioactivity was followed on a Berthold radiotracer equipped with a solid cell.

Quantitation of cytochrome P 450 reductase (cyt P 450 reductase). The NADPH cyt P 450 reductase was measured by its ability to reduce cytochrome c (Vermilion 1978): The reaction was carried out in a double beam spectrophotometer, at room temperature. The reaction mixture contained 0.5 mg of microsomal proteins, 33 µM cytochrome c and 0.05 M phosphate buffer, pH 7.6. The reaction was started by the addition of 0.1 mM NADPH (100 µl of 3 mM solution) to the test cuvette. The final reaction volume was 3 ml. The reaction rate was measured for at least 2 min by the time-dependent increase in absorbance at 550 nm due to the reduction of cytochrome c, using the extinction coefficient $\varepsilon = 21{,}000\ M^{-1}\ cm^{-1}$.

Results

Effect of LtriP treatment on hepatic cyt P 450

The levels of cyt P 450 were monitored for 13 days after LtriP treatment in NMRI mice. A depression was noted from day 1 (32%) until day 4 (40%), after which the levels returned to normal on day 7. A similar observation was made for CCl_4-induced lipid peroxidation, determined by MDA production as already observed[6]. No change occurred in the cyt P 450 reductase activity (table 1). After LtriP treatment, the liver weight was not modified but the amount of hepatic microsomal protein was significantly increased during a period of 15 days (table 2).

The amount of cyt P 450 was higher in male than in female NMRI and C3H/OuJ mice (table 3). One day after LtriP treatment, whatever the sex, a significant decrease (about 40%) in the global cyt P 450 level was observed, as illustrated by a lower ratio of cyt P 450 versus cyt b_5 (even though a sex-dependent variation in cyt b_5 was also observed in NMRI mice). In C3H/HeJ mice, although the quantity of cyt P 450 in females was equivalent to the two other strains cyt P 450 female levels (1 nmol/mg microsomal proteins), the cyt P 450 levels in the males corresponded to only ca. 35% of those of the males of the other two strains (table 3).

Considering the enzymatic activities (E.R.O.D., P.R.O.D., B.R.O.D. and 7-E.C.), no significant difference between the three strains was noted in control mice. B.R.O.D. and 7-E.C. were higher in the females than in the males (fig. 1) when expressed as turnover numbers (nmol/min/nmol cyt P 450). For E.R.O.D. or P.R.O.D. the turnover numbers were very low, regardless of the sex. After LtriP treatment, a significant reduction in E.R.O.D. and 7-E.C. was observed, particularly in male NMRI mice in which 7-E.C. was diminished by about 90%. In contrast, LtriP had no effect on the four enzymatic activities in C3H/HeJ mice in either female or male mice (fig. 1).

Hydroxylation of lauric acid at position C_{11} and C_{12} (11-OH and 12-OH) by hepatic microsomes was deter-

Table 1. Cyt P 450 reductase activity at different time intervals with or without irradiation (7.9 Gy), in hepatic microsomes of female NMRI mice i.p. treated with LtriP (0.5 mg/kg) or saline (controls, C), for 2 consecutive days before irradiation.

Day after treatment with or without irradiation	Cyt P 450 reductase activity nmol/mg protein/min			
	C NI	LtriP NI	C I	LtriP I
1	0.20 ± 0.05	0.22 ± 0.02	0.23 ± 0.03	0.20 ± 0.01
13	0.23 ± 0.01	0.25 ± 0.02	**0.15 ± 0.01**	0.22 ± 0.01
15	0.24 ± 0.02	0.23 ± 0.01	**0.16 ± 0.01**	0.23 ± 0.02
26	0.25 ± 0.01	0.24 ± 0.03	0.23 ± 0.02	0.21 ± 0.01

Number of animals per group = 3; NI or I: non-irradiated or irradiated; bold values were significantly different from non-irradiated controls (C NI) (paired Student's test, $p < 0.05$).

Table 2. Effect of LtriP treatment (i.p., 0.5 mg/kg) over a month on the level of hepatic microsomal proteins and the weight of the liver of NMRI mice, compared to controls (0.5 ml of saline), with or without γ-irradiation (8 Gy).

Day after treatment with or without irradiation	Non-irradiated mice				Irradiated mice			
	controls		LtriP		controls		LtriP	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
1	3.8 ± 0.8	1.41 ± 0.07	**6.9 ± 0.9**	1.49 ± 0.02	**5.8 ± 0.2**	1.37 ± 0.10	**9.8 ± 1.5**	1.53 ± 0.16
8	4.3 ± 0.9	1.60 ± 0.09	**7.5 ± 1.1**	1.43 ± 0.11	**8.6 ± 1.2**	1.37 ± 0.14	**7.7 ± 0.5**	1.53 ± 0.12
15	6.8 ± 0.4	1.54 ± 0.13	**11.0 ± 1.4**	1.43 ± 0.18	**10.2 ± 1.2**	1.32 ± 0.07	**9.6 ± 0.9**	1.38 ± 0.05
18	5.7 ± 0.4	1.40 ± 0.06	6.9 ± 0.7	1.25 ± 0.06	6.8 ± 0.9	1.47 ± 0.05	6.1 ± 0.8	1.43 ± 0.06
30	5.4 ± 0.7	1.51 ± 0.13	6.7 ± 1.1	1.52 ± 0.07	6.8 ± 0.3	1.46 ± 0.14	6.7 ± 1.0	1.55 ± 0.14

(A) = Amount of hepatic microsomal proteins (mg proteins/g liver); (B) = liver weight (g).
Number of animals per group = 3; bold values are significantly different from non-irradiated controls of the corresponding day (paired Student's test, $p < 0.05$).

Table 3. Level of hepatic microsomal cyt P 450 and cyt b_5, one day after treatment with saline (0.5 ml, controls) or LtriP (0.5 mg/kg).

Strain of mice	Sex	Number of animals n		Cytochromes (nmol/mg protein)		cyt P 450
				cyt P 450	cyt b_5	cyt b_5
NMRI	female	12	controls	0.89 ± 0.08	1.05 ± 0.18	0.85
		12	LtriP	**0.51** ± 0.05	0.83 ± 0.2	**0.61**
	male	6	controls	1.5 ± 0.04	0.55 ± 0.06	2.73
		6	LtriP	**0.95** ± 0.03	0.47 ± 0.04	**2.02**
C3H/OuJ	female	6	controls	1.1 ± 0.1	0.55 ± 0.06	2.0
		6	LtriP	**0.64** ± 0.09	0.43 ± 0.05	**1.49**
	male	4	controls	1.73 ± 0.2	0.63 ± 0.04	2.75
		4	LtriP	**1.04** ± 0.01	0.52 ± 0.16	**2.0**
C3H/HeJ	female	4	controls	1.07 ± 0.12	0.63 ± 0.04	1.7
		4	LtriP	0.8 ± 0.1	0.5 ± 0.05	1.6
	male	4	controls	0.57 ± 0.05	0.51 ± 0.07	1.1
		4	LtriP	0.56 ± 0.03	0.5 ± 0.04	1.1

n: number of treated animals per group; bold values are significantly different from their corresponding controls (paired Student's test, $p < 0.05$).

mined for C3H/OuJ and C3H/HeJ strains as a marker of cyt P 450 4A (table 4). In both strains, LtriP treatment decreased the total enzymatic conversion of lauric acid. In male and female C3H/OuJ mice and male C3H/HeJ mice, the 12-OH activity was higher than the 11-OH activity and no significant effect of LtriP was observed on the ratio of 12-OH versus 11-OH activity. A dramatic decrease (74%) in total conversion was observed for female LtriP-treated C3H/HeJ mice with a significant modification of 12-OH versus 11-OH activity, indicating that cyt P 450 4A responsible for 12-hydroxylation of lauric acid was the most affected.

Effect of irradiation on LtriP-treated mice

Survival of the animals in the three different strains. The radioprotection by LtriP, previously observed in female NMRI mice[7], was confirmed in males and shown to be valid for C3H/OuJ mice (fig. 2), with the same turning point period between the 13th and the 15th days. For these two strains, females were more resistant than males to radiation. By contrast, the C3H/HeJ mice (fig. 3) manifested a greater sensitivity to γ-rays, the lethal dose being much lower than that for C3H/OuJ under the same conditions. For female C3H/HeJ, LtriP treatment strengthened the lethality due to irradiation. In contrast, under these experimental conditions, LPS of *Salmonella enteridis* had a significant radioprotective effect on males and females of this strain analogous to that observed for female NMRI mice[7] and male C3H/OuJ mice.

Biological parameters followed in female NMRI mice during evaluation of radiation damage. *1) Microsomal proteins:* The day after irradiation, the amount of microsomal proteins was higher in irradiated animals than in control animals (table 2) and the lipopeptide treatment led to an additional increase. This supplementary effect was not observed on days 8 and 15. The increase in the level of microsomal proteins was observed up to day 15 and then returned to the value of the non-irradiated control on day 18 in surviving animals.

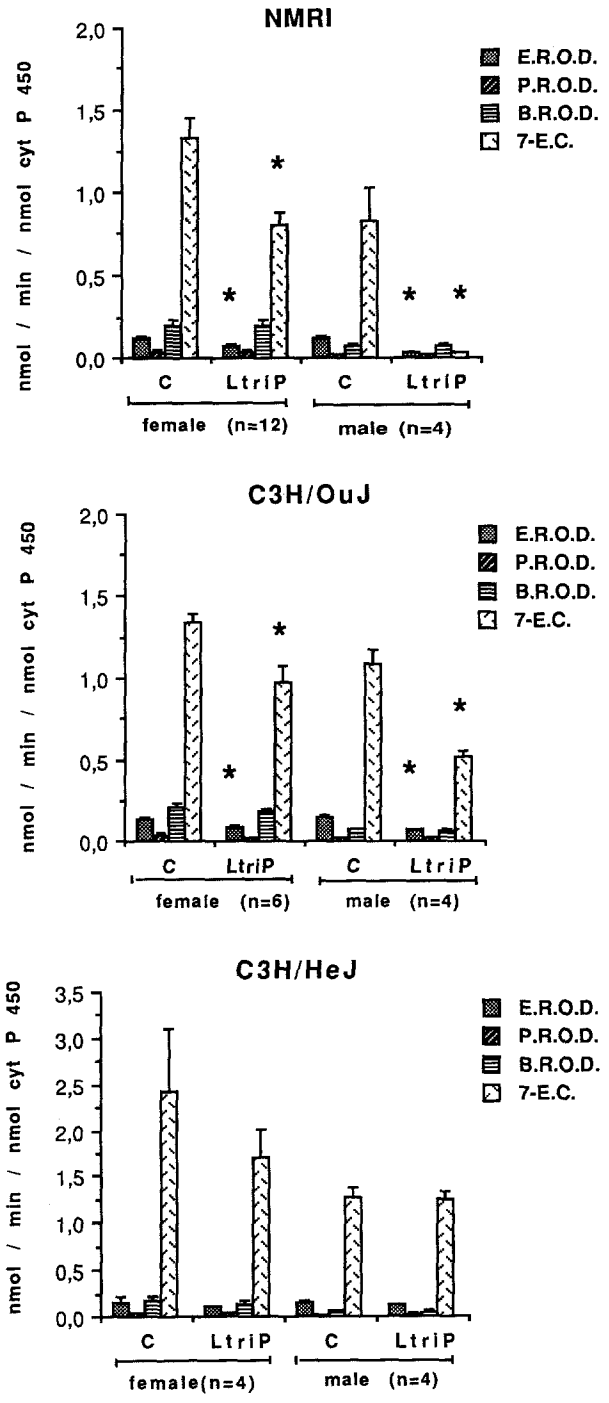

Figure 1. Enzymatic activities (E.R.O.D., P.R.O.D., B.R.O.D. and 7-E.C.) in hepatic microsomes of NMRI, C3H/OuJ and C3H/HeJ mice, one day after the last treatment with saline (0.5 ml, C: controls) or LtriP (0.5 mg/kg).
n = number of animals per group; * = significantly different from controls (paired Student's test, $p < 0.05$).

2) Cyt P 450 level and enzymatic activities: For irradiated controls, if no decrease in cyt P 450 and enzymatic activities was detectable at day 1, the levels of cyt P 450 and cyt b_5 as well as E.R.O.D., B.R.O.D. and 7-E.C. activities were significantly reduced at day 13 compared to non-irradiated animals (fig. 4). By contrast, for LtriP-treated animals, if the irradiation did not modify the decreasing effect of the lipopeptide observed at day 1, at day 13 a total protection against γ-ray damage to the cyt P 450 level and enzymatic activities was observed (fig. 4). The same protection was observed for the cyt P 450 reductase activity which decreased after irradiation in control animals but not in LtriP-treated mice (table 1).
3) Comparison with the effects of IL-1 and TNFα: Since IL-1 and TNFα, two radioprotective cytokines, are secreted by macrophages after LtriP stimulation[7], the influence of these two immunological factors on the expression of cyt P 450, 13 days after injection, was studied in hepatic microsomes of irradiated female NMRI mice. As observed with LtriP treatment, at day 13 after irradiation the amounts of cyt P 450 of irradiated IL-1-treated mice were similar to those of non-irradiated mice. In contrast TNFα did not protect cyt P 450 against radiation damage as a lower level of cyt P 450 was observed.

Discussion

LtriP effects on cyt P 450 and radioprotection: possible relationship to the cytokine induction. After LtriP treatment of NMRI and C3H/OuJ strains, a decrease in the hepatic microsomal cyt P 450 level was observed which may be related to the significant increase in the level of total liver proteins. At the same time, some of the enzymatic monooxygenase activities such as E.R.O.D. and 7-E.C., expressed per nmol of cyt P 450 and therefore independent of the amount of total proteins, were reduced while the enzymatic activity of cyt P 450 reductase was not modified. These results mimicked those obtained with equivalent doses of endotoxins in CBA mice[28]. It should be noted that IL-1, the cytokine induced by endotoxins and the lipopeptide, caused a decrease in these two cyt P 450 activities in rat fetal hepatocytes[29]. Furthermore, as reported in the literature, IL-1[18] and TNFα[30] mediated the same effects not only in C3H/OuJ (lps^n/lps^n) but also in C3H/HeJ (lps^d/lps^d) mice. However in this latter strain, no modification of B.R.O.D. and 7-E.C. was observed after LtriP treatment. This may be related to the fact that, like the lipid A part of LPS, LtriP did not stimulate secretion of IL-1 by C3H/HeJ macrophages (results not shown). LtriP and lipid A seem to act on hepatic monooxygenase activities by a common pathway which involves the monokines. Intense protein synthesis was observed after LtriP treatment, which may reflect stimulation of the synthesis of acute phase proteins with proinflammatory cytokines such as IL-1, TNFα and IL-6. In fact, among microsomal proteins we observed an increased expression of transferrin[31] which has been reported to be a positive acute phase protein in mice[32].

Table 4. 11- and 12-hydroxylations of lauric acid within hepatic microsomes of male and female C3H/OuJ and C3H/HeJ mice, one day after 2 successive daily treatments with saline (500 µl/animals, controls) or LtriP (0.5 mg/kg).

	Total conversion of lauric acid nmol/min/mg protein	11-OH conversion % of total conversion	12-OH conversion % of total conversion	12-OH/11-OH
C3H/OuJ male				
Controls	51.2 ± 11.2	40.6 ± 1.8	59.4 ± 1.8	1.5 ± 0.1
LtriP	**26.8 ± 3.2**	37.4 ± 1.4	62.6 ± 1.4	1.7 ± 0.1
C3H/OuJ female				
Controls	36.4 ± 4.6	40.1 ± 1.9	59.9 ± 1.9	1.5 ± 0.1
LtriP	30.2 ± 3.4	37.4 ± 1.6	62.6 ± 1.6	1.7 ± 0.1
C3H/HeJ male				
Controls	28.6 ± 0.6	32.0 ± 1.3	68.0 ± 1.3	2.1 ± 0.2
LtriP	**23.6 ± 1.6**	34.3 ± 1.6	65.7 ± 1.6	1.9 ± 0.1
C3H/HeJ female				
Controls	60.2 ± 1.6	25.8 ± 1.4	74.2 ± 1.4	2.9 ± 0.2
LtriP	**15.6 ± 2.2**	**52.8** ± 3.0	**47.2** ± 3.0	**0.9** ± 0.1

Bold values are significantly different from corresponding control values (paired Student's test, $p < 0.05$, number of animals per group = 3).

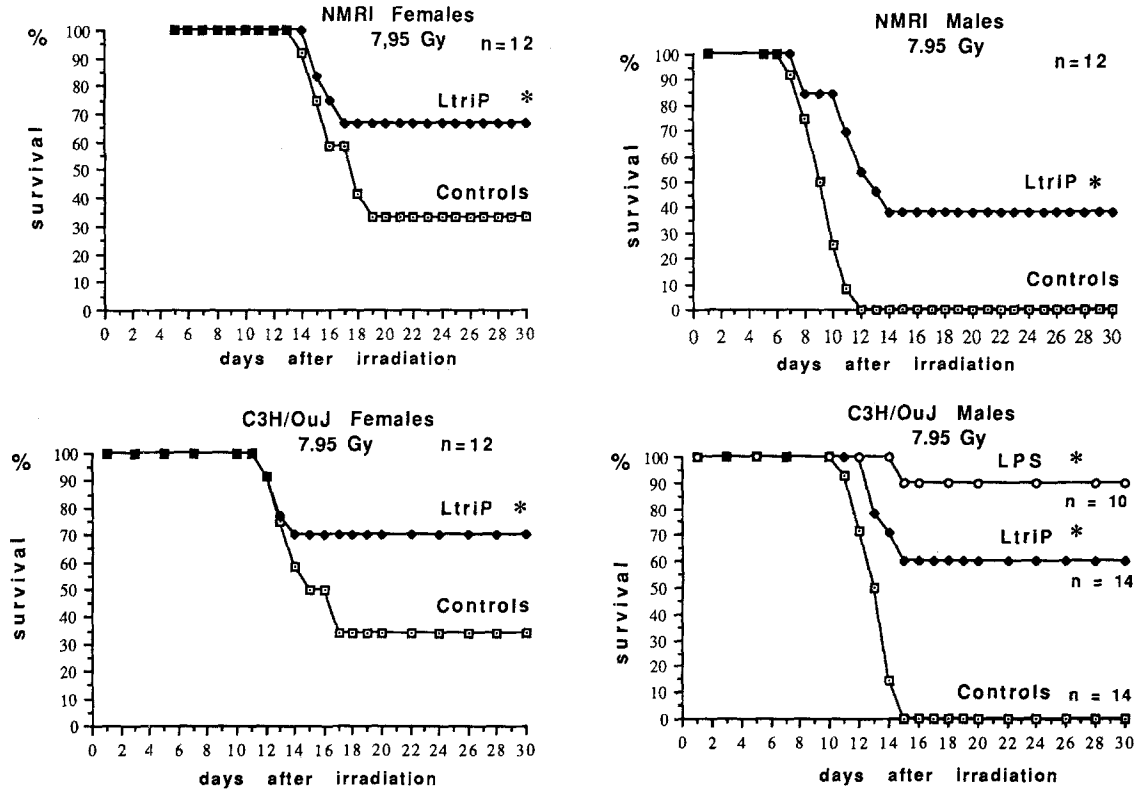

Figure 2. Survival after irradiation of female and male NMRI and C3H/OuJ mice, i.p. treated for 2 consecutive days before irradiation with saline (0.5 ml, controls), LtriP (0.5 mg/kg) or LPS (2 µg/animal).
n = number of animals per group; * = significantly different from controls (Logrank test, $p < 0.05$).

Cytokines may also be implicated in the radioprotective effect of the lipopeptide. In irradiated control animals, an increased synthesis of proteins was observed just after irradiation which persisted for approximately 15 days. The result is in agreement with the report of induced multiple protein synthesis in human cells after ionizing radiation[33]. Synthesis of immunological factors such as IL-1 and TNFα has also been described[34,35], which may represent part of the host defence mechanism mediated by these radioprotective cytokines[36]. When animals were pretreated with LtriP and irradiation was performed on the first day after LtriP treat-

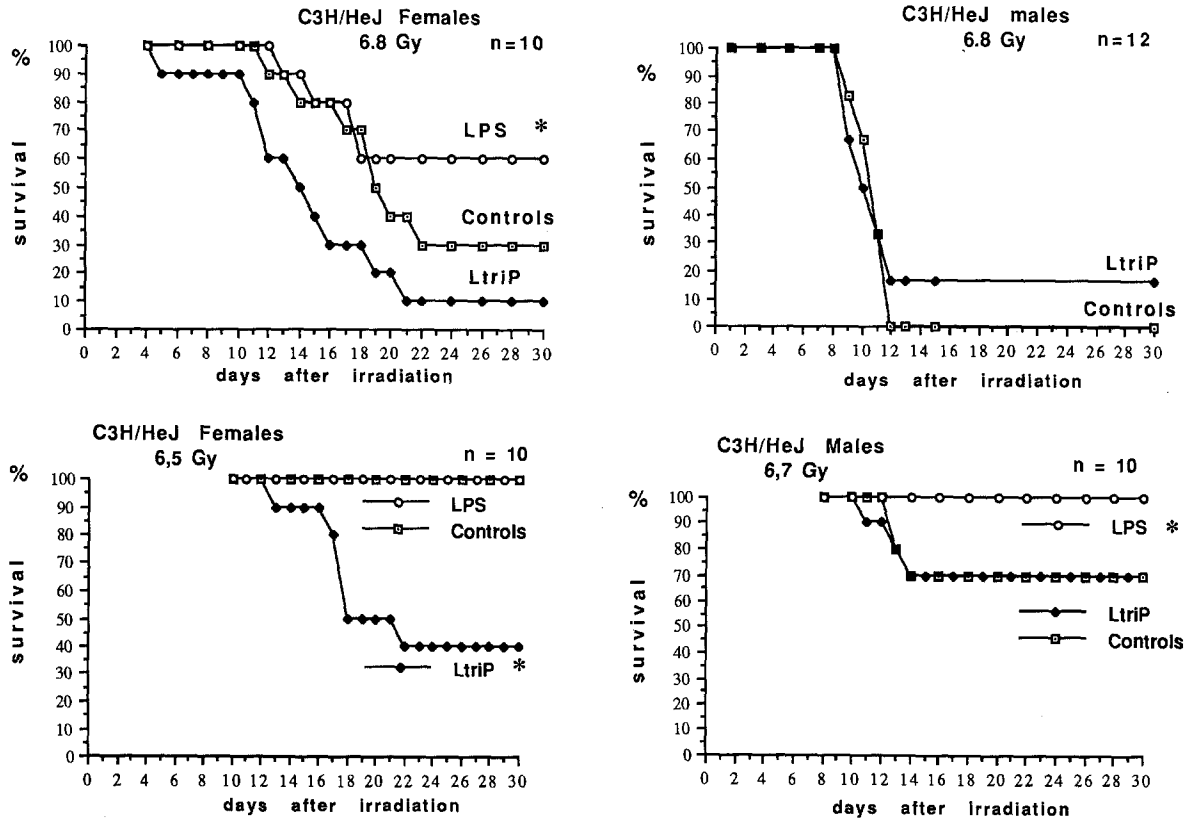

Figure 3. Survival after irradiation of female and male C3H/HeJ mice, i.p. treated for 2 consecutive days before irradiation with saline (0.5 ml, controls), LtriP (0.5 mg/kg) or LPS (2 µg/animal).
n = number of animals per group; * = significantly different from controls (Logrank test, $p < 0.05$).

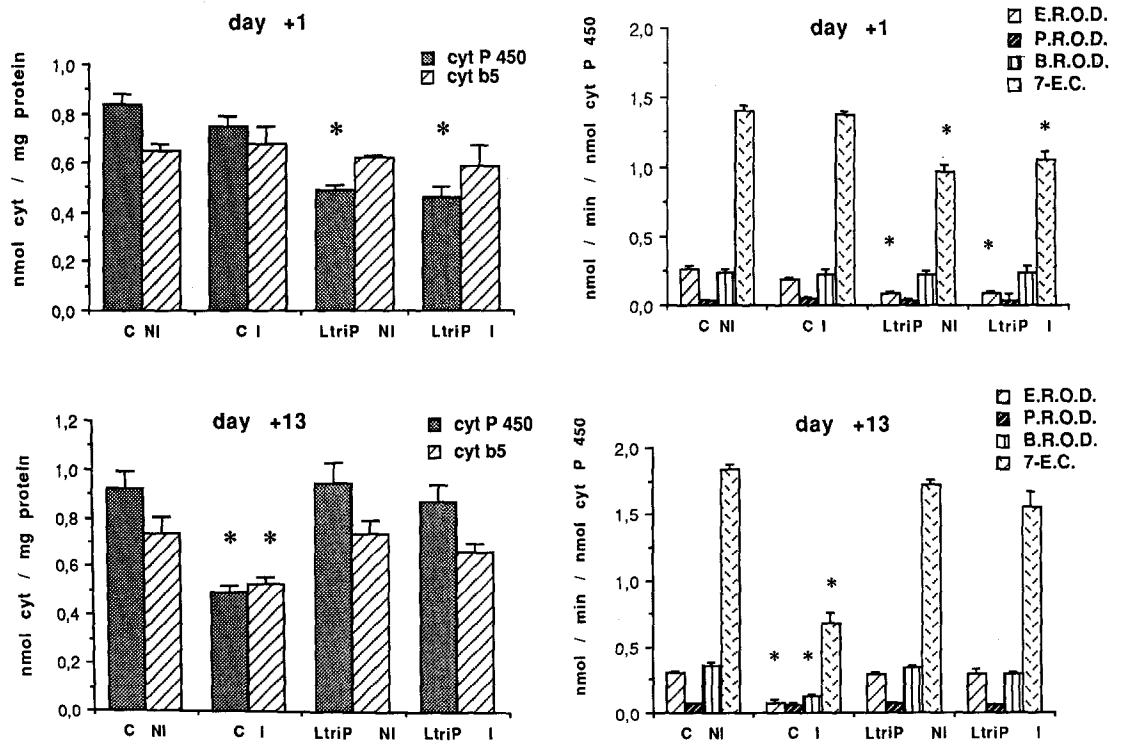

Figure 4. Effect of LtriP (0.5 mg/kg) compared to controls (C, 0.5 ml saline) on the amount of cyt P 450 and cyt b_5 and enzymatic activities of hepatic microsomes of female NMRI mice at day +1 and day +13 after irradiation (8 Gy).
Number of animals per group = 6; NI = non-irradiated; I = irradiated; * = significantly different from non-irradiated controls (paired Student's test, $p < 0.05$).

ment, the two effects on the synthesis of proteins were additive. To obtain maximum lipopeptide activity, two successive daily injections were required, suggesting the synthesis of new mediators. LtriP might prepare the animals by anticipating the cytokine defensive response that can be amplified by γ-ray treatment. The protective effect of the lipopeptide against deleterous radiation damage was observed starting from the 13th day onwards, with total preservation of the amount of cyt P 450 and the specific monooxygenase activities in LtriP-treated but not in non-treated mice. We also observed protection of these hepatic enzymes after IL-1 treatment or IL-1 and TNFα cotreatment, supporting the idea that monokine induction is involved in the lipopeptide radioprotection.

LtriP activities and sex-dependence. In connection with the higher sensitivity of male mice to radiation, we have compared the expression of five cyt P 450 activities in male and female mice of each strain investigated. We have found that B.R.O.D. and 7-E.C. were lower in males than in females, but the radioprotection exerted by LtriP was sex-independent in C3H/OuJ and NMRI and may not be related to these two cyt P 450 activities because the lipopeptide did not modify B.R.O.D. and led to a marked decrease of 7-E.C. in male NMRI. A hormonal control of radioprotection has been described for the rat[37] and mouse[38] treated with steroids. Recently, IL-1 and LPS were reported to have a sexually dimorphic effect on the hypothalamo-pituary-adrenal axis in several mouse strains[39]. This may also be the case with C3H/HeJ mice for which LtriP was not significantly radioprotective for males and, furthermore, clearly increased the sensitivity of females to irradiation. This effect, accompanied by the dramatic decrease of lauric acid ω-hydroxylases in female C3H/HeJ, suggests that in the absence of IL-1 and TNFα induction, LtriP may control specific cyt P 450 isozyme induction such as cyt P 450 4A. Actually, these cyt P 450 isozymes are fatty acid ω-hydroxylases which oxidize the terminal methyl group of saturated and unsaturated fatty acids, including radioprotector derivatives such as the prostaglandins, thromboxanes and prostacyclins[40]. Some of these compounds were described as radioprotectors[16]. Any modification of the metabolism of these biolipids may influence their physiological role. It is to be noted that the locus of cyt P 450 4A, which is responsible for ω-hydroxylase activities, is situated close to the *Lps* locus on mouse chromosome 4[41], which differentiates C3H/OuJ from C3H/HeJ mice in the macrophage response to lipid A of gram-negative bacterial endotoxins. This may be related to the observation that induction of cyt P 450 4A by clofibric acid in cultured fetal rat hepatocytes is inhibited by IL-1[42].

Another pathway of endotoxin radioprotection which is independent of that of lipid A may be suggested. It is worth noting that while LtriP increased radiosensitivity in female C3H/HeJ mice and was without protective activity for males of this strain, the LPS of *S. enteriditis* had a protective effect regardless of the sex of the mouse (fig. 3). This may be due to the low percentage of proteins reported to be present in this LPS preparation. The lipid A-associated proteins were shown to serve as a 'trigger' signal by primed C3H/HeJ macrophages which did not recognize lipid A[21]. Recently, Neta et al. showed that the stem cell factor, the ligand of the receptor for hemopoietic cytokine (c-kit), acts synergistically with IL-1 in the radioprotection of mice[43].

A role in protection against oxidative damage for a cyt P 450 induced in yeast by irradiation was suggested by Morichetti et al.[44]. Our results do not conclusively implicate the hepatic cyt P 450 activities considered in this study in the protection of mice against radiation. However, they might exert an indirect role via their involvement in steroid metabolism[45] since steroid hormones have been implicated in radioprotection[37]. Furthermore, a role for hemoproteins in this phenomenon is supported by the recent finding of a synergistic effect of heme and IL-1 on stromal regeneration after irradiation[46]. The complexity of hemoproteins, which play a large role in metabolism of free radicals and biolipids, offers wide opportunities for research in the field of radioprotection.

Abbreviations

A_2pm: diaminopimelic acid; **BCG**: Bacillus Calmette Guerin; **B.R.O.D.**: benzyloxyresorufin-O-dealkylase; **cyt**: cytochrome; **DMSO**: dimethylsulfoxide; **7-E.C.**: 7-ethoxycoumarin-O-deethylase; **EDTA**: ethylenediaminetetraacetic acid; **E.R.O.D.**: ethoxyresorufin-O-deethylase; **IL-1**: interleukin-1; **i.p.**: intraperitoneal; **LPS**: lipopolysaccharide or endotoxin; **LtriP**: lauroyltripeptide; **M-CSF**: macrophage-colony stimulating factor; **MDA**: malondialdehyde; **NADPH**: reduced β-nicotinamide adenine dinucleotide phosphate; **P.R.O.D.**: pentoxyresorufin-O-dealkylase; **TNFα**: tumor necrosis factor α.

Acknowledgments. We thank Prof. P. Roubertoux for helpful discussions, Prof. P. Ortiz de Montellano and Dr. S. Booker for kindly revising the English style of the manuscript and M. Jaouen for her excellent technical assistance.
Authorisation from the French Ministère de l'Agriculture et de la Forêt to experiment on animals: n° 04861, Dr. D. Migliore-Samour.

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