Effect of IFN-α on $CC1_4$-Induced Fibrosis of the Liver and Immune Status in Mice of Different Age

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In young, adult, and old mice fibrosis was induced by administration of $CC1_4$ and treated with IFN-α. Liver fibrosis was evaluated by morphometry of argyrophilic fibers, immune status by the splenocyte proliferative response. Minimum immunosuppression and maximum antifibrotic effect were observed in young mice, while adult mice exhibited pronounced immunotoxicity and weak response to interferon therapy.

Key Words: *$CC1_4$; fibrosis; age; IFN-α*

The consequences of exposure to toxic substances in children and adults can differ because of specific features in their physiology, metabolism, pharmacokinetics, diets [6]. These differences can include the dynamics of liver fibrosis development induced by chronic $CC1_4$ intoxication and response to antifibrotic therapy, which is little studied in young age, both experimentally and clinically.

MATERIALS AND METHODS

In group 1 fibrosis was induced by intraperitoneal administration of $CC1_4$ in ascending doses (0.05-0.50 ml 10% solution) for 7 weeks to male C57Bl/6 mice of young (6-7 weeks), adult (8-12 months), and old (2 years) age. Lower doses of $CC1_4$ (0.05-0.10 ml 10% solution) were used in young mice because of high mortality. After induction of fibrosis the animals of groups 2 and 3 (the same age subgroups) were daily injected with distilled water for 3 weeks (sham treatment; 0.5 ml/mouse) or IFN-α (Vektor-Farm, 1000 U/mouse), respectively. Adult mice served as intact controls. Histological preparations of the liver were examined in order to determine the stage of fibrosis (0-4 points; hematoxylin and eosin), estimate the total volume density of argyrophilic fibers (VDAF; morphometry, silver impregnation). Splenocytes were cultured in round-bottom plates (Linbro) in a concentration of 300×10^3/well at 37°C in an atmosphere consisting of 5% CO_2 and 95% air in RPMI-1640 with 10% FCS, 2 mM L-glutamine, and 4×10^{-5} M 2-mercaptoethanol. The cells were stimulated with mitogens: Con A and *E. coli* 055:B5 LPS (Sigma) in doses of 2 and 30 μg/ml, respectively. Splenocyte proliferation was evaluated by incorporation of ^{3}H-thymidine. The results were statistically processed using nonparametric tests (χ^2, Mann—Whitney) at $p<0.05$.

RESULTS

In group 1 injection of $CC1_4$ led to the development of septal fibrosis or cirrhosis in all animals, more severe in adult animals (Table 2). VDAF was the same in young and adult mice, despite the use of lower $CC1_4$ dose in young animals (Table 1). Fibrotic process was paralleled by suppression of spontaneous and LPS-stimulated splenocyte proliferation, which attests to an immunosuppressive effect of $CC1_4$ (Table 3). The suppression of proliferative response was maximum in adult mice, while in young and old mice T-cell response was not suppressed (Con A-stimulated proliferation).

In group 2 appreciable reduction of fibrosis compared to untreated mice was observed only for adults

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TABLE 1. Total Volume Density of Argyrophilic Fibers in the Liver (%)

Group	Initial	Experimental conditions		
		$CC1_4$	$CC1_4+H_2O$	$CC1_4$+IFN
Intact	9.3			
Young	—	13.1 ($p=10^{-6}$)*	13.6 ($p=0.027$)$^{++}$	11.7 ($p=0.044$)**
Adult	—	13.1 ($p=0.0004$)*	19.2 ($p=10^{-8}$)**	17.8 ($p=10^{-5}$)**
Old	—	9.8 ($p=0.045$)***	13.1 ($p=0.001$)**; ($p=3*10^{-5}$)$^{+}$	9.8 ($p=0.0001$)$^{+++}$; ($p=0.04$)^{o}

Note. The data are presented as a median; *p*: compared to *intact, **untreated of the same age, ***untreated adult, $^{+}$sham-treated adult, $^{++}$IFN treated young, $^{+++}$IFN treated adult, ^{o}sham-treated old animals.

(Table 2). VDAF in young animals did not change in comparison with untreated ones and increased in adult and old mice by 47 and 34%, respectively (Table 1). VDAF in group 2 old animals was lower than in adult animals by 47%, while in young animals receiving $CC1_4$ in lower doses this parameter was comparable to that in old mice, but was lower than in adult mice by 41% ($p=3.4\times10^{-6}$). This indirectly attests to pronounced liability of young animals to fibrosis. Sham treatment was associated with further inhibition of splenocyte proliferation, more pronounced in young and old mice (Table 3) compared to untreated animals; the absolute values were minimum in adult mice, which was associated with maximum level of liver fibrosis (Tables 1, 2).

Decrease of fibrosis stage (Table 2) and VDAF regression (by 11%; Table 1) were detected in group 3 young mice in comparison with untreated animals of this age groups; that is, two methods for evaluation of fibrosis level gave similar results. VDAF in young mice was 14% lower after interferon therapy, while the levels of spontaneous and LPS-stimulated splenocyte proliferation were higher than in sham-treated animals. Interferon therapy was less effective in old animals: fibrosis stage decreased in 100% animals of this subgroup (Table 2), but VDAF remained at the same level as in untreated mice (Table 1). Proliferative response decreased in comparison with groups 1 and 2 (Table 3). The effect of IFN therapy in old mice manifested in a 25% decrease of VDAF in comparison with group 2 animals of the same age. The treatment was minimum effective in adult animals; it was associated with maximum immunosuppression among all age subgroups. Fibrosis stage decreased after treatment, but septal fibrosis persisted in some animals (Table 2), while VDAF even increased (by 36%) in comparison with untreated animals and did not differ from that in group 2 animals of the same age, this indicating poor response to therapy.

Immune mechanisms play an important role in the pathogenesis of $CC1_4$-induced fibrosis. TNF-α is believed to play the key role in hepatocyte damage; it is noteworthy that injection of $CC1_4$ does not lead to liver fibrosis in mice genetically lacking receptors for this cytokine [14]. The tempo of $CC1_4$ fibrosis development is appreciably higher in mice with the genetic predominance of type 2 T-helper response (BALB/c) in comparison with type I T-helper response (C57Bl/6) [13]. Therapy with IFN-α impaired the production of TNF-α [5] and of other proinflammatory cytokines [15] and led to direct suppression of collagenogenesis in mice with $CC1_4$ fibrosis of the liver because of collagen gene transcription blockade [7]. The antifibrotic effect of IFN-α, presenting as decreased count of collagen-producing cells, was observed during treatment of $CC1_4$ fibrosis in rats [10]; the effect of therapy can be improved, if IFN-α treatment is started early and lasts for a long time [4]. In humans

TABLE 2. Distribution of Fibrosis Stages in Animals

Group	Experimental conditions		
	$CC1_4$	$CC1_4+H_2O$	$CC1_4$+IFN
Control	(5-0-0-0-0)		
Young	0-0-1-2-2 ($p=0.005$)*	0-0-4-2-0	0-0-6-0-0 ($p=0.011$)**
Adult	0-0-0-0-5 ($p=0.003$)*	0-0-0-6-0 ($p=0.002$)**	0-0-3-3-0 ($p=0.002$)**
Old	0-0-0-3-2 ($p=0.005$)*	0-0-1-3-1	0-0-5-0-0 ($p=0.005$)**

Note. The data are presented as the number of animals with the corresponding stage of fibrosis: 0-1-2-3-4. *p*: compared to *control, **untreated animals of the same age.

TABLE 3. Splenocyte Proliferation in Mice of Different Age

Group (number of animals)		Splenocyte proliferation (cpm)		
		spontaneous	LPS stimulated	Con A stimulated
Intact (n=36)		5550±2200	21 700±4900	26 500±4600
1	young (n=15)	3200±1300 (p=0.003)*	18 600±3000 (0.06)*	39 000±7000 ($p=10^{-6}$)*
	adult (n=15)	1200±380 ($p=3\times10^{-8}$)*	13 700±3700 ($p=7\times10^{-6}$)*	17 000±4500 ($p=5\times10^{-6}$)*
	old (n=15)	2400±1350 ($p=7\times10^{-5}$)*; (p=0.002)***	21 600±3800 ($p=10^{-5}$)***	31 400±7400 ($p=10^{-6}$)***
2	young (n=18)	1100±660 ($p=2\times10^{-6}$)**	13 500±4000 (p=0.0001)**	27 900±13 100 (p=0.012)**
	adult (n=18)	390±240 ($p=1.4\times10^{-8}$)***	12 100±7900	18 200±8100
	old (n=12)	810±620 (p=0.0002)$^{+}$	15 200±2600 ($p=10^{-4}$)$^{+}$	15 200±4500 ($p=10^{-6}$)$^{+}$; (p=0.043)$^{+++}$
3	young (n=18)	2400±1200 (p=0.05)**; (p=0.002)$^{++}$	17 800±4200 (p=0.005)$^{++}$	30 000±10 300 (p=0.015)**
	adult (n=15)	280±170 ($p=3\times10^{-8}$)***	17 000±5900	10 900±4800 (p=0.002)***$^{+++}$
	old (n=15)	660±580 ($p=5\times10^{-6}$)$^{+}$	20 400±12 200	11 100±7500 ($p=4\times10^{-7}$)$^{+}$

Note. The data are presented as arithmetic mean±standard deviation; p: compared to *control; **untreated young mice; ***untreated adult mice; $^{+}$untreated old mice; $^{++}$sham-treated young mice; $^{+++}$sham-treated adult mice.

IFN-α therapy of hepatitis leads to an antifibrogenic effect [15], presumably, because of suppressed expression of proinflammatory cytokines, including TNF-α [5], and reduction of fibroblast-stimulating effects.

$CC1_4$ is characterized by an immunotoxic effect suppressing the proliferative response of lymphocytes (LPS and Con A stimulated) and phagocytosis and NK activity [9]. Suppression of mainly T cell activity after 7-day administration of $CC1_4$ [3] is in line with our findings. Our results demonstrated age-associated differences in the formation of liver fibrosis and immune status during interferon therapy: the severity of immune disorders is lower and antifibrotic treatment is more effective in young animals, despite significant fibrosis (with consideration for the lower dose) than in animals of older groups. The immunotoxic effect of $CC1_4$ was the highest in adult animals, which was associated with the maximum level of fibrosis.

Young adult rats demonstrate more severe involvement of the liver after $CC1_4$ injection than old rats [12]. Acute $CC1_4$ poisoning of suckling rats results in more severe necrosis than in adult rats because of more intense LPO processes [1,8]. Newborn rats develop $CC1_4$-induced cirrhosis more rapidly than 12-week-old ones [11]. Newborn and young rats are more resistant to hepatotoxins than adult animals due to a higher level of hepatocyte regeneration [2]. Our results indicate that 6-7-week-old mice respond by appreciable fibrosis to the hepatotoxin, but if the treatment is started early, regression of fibrosis is possible age. The mechanisms of hepatotoxin effect in young animals with immature immune response and metabolic systems of the liver deserve further studies for the correction of therapeutic approaches.

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