Perftoran as a Means Modulating the Functional Activity of Liver Macrophages

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Experiments on mice showed that perfluorocarbon emulsion Perftoran modulated phagocytic activity of liver macrophages (evaluated by LD_{50} for *Salmonella typhi* endotoxin and the rate of elimination of Chinese ink particles from the bloodflow). Phagocytic activity was suppressed for 3 days after injection of 10 mg/kg emulsion, but then increased above the control. Perftoran had a favorable impact on the course of experimental hepatitis in a model experiment based on hyperactivity of Kupffer cells. Perftoran virtually prevented the development of severe hepatitis after prophylactic injection and notably attenuated hepatocyte cytolysis when used as therapeutic mean.

Key Words: *Kupffer cells; phagocytosis; perfluorocarbons; Perftoran; experimental hepatitis*

Reaction of the mononuclear phagocyte system (MPS), in particular, liver macrophages, plays an important role in the pathogenesis of liver inflammation in viral hepatitis B. The severity of mesenchymal inflammatory and cytolytic syndromes directly depends on the reactivity of liver macrophages (Kupffer cells, KC). Severe hepatitis is associated with hyperactivation of KC and hyperproduction of active cytotoxic substances by these cells [2,9]. Autoaggression is directed towards not only infected hepatocytes, but also intact liver cells. KC hyperreaction is hazardous for the organism and requires therapeutic intervention aimed at regulation of their activity. The known effects of perfluorocarbon emulsions on phagocytic activity [3,7] prompted us to investigate these properties experimentally in order to evaluate the possibility and efficiency of clinical use of perfluorocarbons in severe forms of hepatitis B.

MATERIALS AND METHODS

Intoxication caused by typhoid fever in albino mice was selected as the first experimental model. The liver plays the key role in elimination of endotoxin from the blood. Up to 80% endotoxin is absorbed by the liver 15 minutes after its intravenous injection [8].

Salmonella typhi endotoxin ty-4446, lot 158 (St. Petersburg Institute of Vaccines and Sera) in 0.9% NaCl was injected to albino mice intraperitoneally in increasing doses (2-80 mg/kg) 1 h, 1, 3, and 6 days after intraperitoneal injection of Perftoran (PF) emulsion in a dose of 10 ml/kg. Controls were injected with the same volume of 0.9% NaCl. The animals were observed for 3 days; some animals died during this period. LD_{50} was evaluated by the method of least squares.

In the second experimental series the effect of PF on the clearance of ink particles was evaluated. It should be noted that ink particles were phagocytosed by liver macrophages.

Ink particles were injected intravenously (0.01 ml) 1 h, 1, 3, and 6 days after intravenous PF (10 ml/kg). Each experimental group consisted of 4-5 animals. Blood (0.025 ml) was collected from the retroorbital sinus every 2 min for 15 min after ink injection and mixed with 2.0 ml 0.1% sodium carbonate. After hemolysis the presence of ink in the samples was evaluated by calorimetry at λ=675 nm. The results were expressed in the arbitrary ink clearance units by the formula:

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$$\frac{\lg C_1 - \lg C_2}{T_2 - T_1},$$

where C_1 and C_2 are ink concentrations during the first (T_1) and last (T_2) measurements [4].

Acute hepatitis was induced as described previously [5] with some modifications [1]: the rats intravenously received heat-inactivated *Propyonibacterium acnes* (10^{10} cells/ml 0.9% NaCl) and after 6 days were injected with *S. typhi* endotoxin (1.5 mg/kg), i.e. the resolving sublethal dose of endotoxin was administered at the peak of KC activation by *P. acnes* culture. This leads to hyperactivation of liver macrophages, and hepatitis develops as a result of hepatocyte damage with proinflammatory agents of macrophagal origin. A similar picture was observed in humans with acute viral hepatitis B.

The rats were divided into 6 groups, 18 animals per group. Experimental rats of groups 1 and 2 received PF one day before or after the toxin, respectively; group 3 rats received 3 injections of PF: 1, 2, and 3 days after challenge with the toxin. The animals of three control groups received the same doses of rheopolyglucin at the same terms. PF and rheopolyglucin were injected into the caudal vein in a dose of 10 ml/kg.

The efficiency of PF was evaluated by the dynamics of serum alanine aminotransferase (AlAT) activity on days 2, 4, and 6 of hepatitis.

RESULTS

Symptoms of intoxication (inertia, food refusal, etc.) developed for 12 h after injection of endotoxin. The fur was shuffled, some animals had liquid stool. The severity of intoxication increased with increasing the doses of the toxin. The animals died mainly within the first 24 h after challenge with the toxin.

One hour after injection of PF, the sensitivity to endotoxin increased and remained high for 3 days (Table 1). The maximum decrease of LD_{50} (3.7 times) was observed 1 day after PF injection. On day 7 after injection of PF the sensitivity to endotoxin notably decreased and LD_{50} increased more than 10-fold (Table 1).

In experiments with endotoxin we indirectly evaluated activity of KC (LD_{50} of *S. typhi* LPS for mice); PF was injected intraperitoneally, which ensured its maximum delivery to the liver via the portal blood flow. These data were to be verified by direct methods for evaluation of phagocytosis activity with intravenous injection of the agent.

The time course of PF effect on the clearance of ink particles was identical to its effect in experiments with endotoxin (Table 1), but it is noteworthy that MPS blocking after intravenous injection of PF was less lasting.

TABLE 1. Effect of PF on LD_{50} of *S. typhi* Toxin and Clearance of Chinese Ink Particles in Albino Mice ($M \pm m$)

Experiment conditions	LD_{50}, mg/kg	Ink clearance, arb. units
Control	30.7±3.4	0.172±0.021
Time after PF injection		
1 h	20.5±5.6*	0.129±0.022*
1 day	8.2±1.7*	0.055±0.014*
3 day	14.2±2.6*	0.158±0.020
6 day	327.0±36.7*	0.280±0.057*

Note. *$p<0.05$ compared to the control.

By day 3 after PF injection clearance of ink particles virtually did not differ from that in the control; after this the process of ink clearance became more rapid, reaching the maximum on days 4-5. The parameters started to normalize from day 6.

The results of phase I of experiments showed that PF can be used for modulating activity of liver macrophages, but it was necessary to elucidate whether massive phagocytosis of PF particles can induce hyperactivation of macrophages with its untoward consequences, which was done in rat experiments.

The rats developed severe hepatitis after resolving dose of endotoxin. The animals were inert, did not move, refused from food. Some animals died within the first 24 h after hepatitis modeling. The data presented in Table 2 show mortality in different groups (each experimental and control group consisting of 18 animals at the start of the experiment). We should like to emphasize that the rats died before, but not after PF treatment in both control and experimental groups which received therapeutic (after endotoxin challenge) injection of PF or rheopolyglucin.

After injection of the resolving dose of endotoxin control animals developed severe hepatitis, associated with a drastic (17-19 times) increase in AlAT activity (Table 2). This parameter did not returned to normal even after 6 days. The course of hepatitis was more benign in animals receiving preventive injections of PF. Peak activity of AlAT was almost 2-fold lower than in the corresponding controls and after 4 days did not differ from normal (Table 2).

The therapeutic effect of single injection of PF was less pronounced: on day 2 of hepatitis AlAT activity virtually did not differ from that in the corresponding control group, but later this parameter returned to normal more rapidly, and by day 6 it did not differ from normal (Table 2). AlAT activity more rapidly returned to normal in rats receiving 3 injections of PF (compared to rats receiving one injection).

Hence, the effect of PF on mouse resistance to LPS is phase-dependent. This can be explained by the

TABLE 2. Effect of PF on Serum AlAT Activity (U/liter) in Rats with Experimental Hepatitis (*M*±*m*)

Experiment conditions		Time after endotoxin injection, days		
		2	4	6
Pretreatment with PF	control (*n*=15)	1085.4±112.3	359.5±24.4	127.8±18.1
	experiment (*n*=18)	612.4±38.2*	74.8±14.1*	58.5±10.2*
Injection of PF after toxin				
single	control (*n*=14)	1174.5±125.9	376.6±28.3	140.4±20.5
	experiment (*n*=13)	1015.4±94.8	223.5±20.6*	66.2±14.0*
three	control (*n*=15)	1029.5±82.6	312.8±24.4	154.7±18.8
	experiment (*n*=15)	911.4±73.3	119.6±16.4*	60.2±9.9*

Note. *$p<0.05$ compared to the corresponding control. AlAT activity in intact animals 63.0±12.7 U/liter.

influence of PF on endotoxin binding by KC and endotheliocytes in the liver. Liver macrophages loaded with PF particles loose their capacity to phagocytose LPS molecules and endotoxin crosses the hepatic barrier and appears in the circulation causing generalized toxic injuries and death. The duration of inhibition of phagocytic activity depends on the time needed for the appearance of new cells replacing PF-loaded phagocytes. We believe that this pool is replenished due to activation of hemopoiesis in the bone marrow and mobilization of reserves from depots. Presumably, the response is excessive and leads to accumulation of numerous phagocytes in the liver, so that the total capacity of the phagocytic pool surpasses the *status quo*. This can explain increasing resistance of animals to endotoxin observed 6 days after PF administration.

PF did not aggravate the course of severe hepatitis, *i. e.* phagocytosis of PF particles by liver macrophages was not associated with enhanced release of proinflammatory cytokines by these cells. Preliminary blockade of MPS induced by PF prevented hyperactivation of macrophages in response to endotoxin, while injection of PF at the peak of hepatitis attenuated the damaging effect of KC on hepatocytes. Repeated injection of PF with one-day intervals potentiated the therapeutic effect of the preparation.

Biphasic reaction of the phagocytic system to perfluorocarbon emulsions allows purposeful modification of its activity depending on the clinical situation. Hyperactivation of macrophages (autoimmune vasculitis, pulmonary distress syndrome, severe infections) necessitates repeated massive infusions of the emulsion at short (up no more than 24 h) intervals in order to prevent secondary activation of MPS. The same principle is to underlie treatment protocols for infections when the agent uses macrophages for its own reproduction, for example, in HIV infection. We recommend infusions of the emulsion with longer intervals (with consideration for the duration of the secondary activation phase) in diseases requiring activation of the phagocytic system (chronic infections, cancer, *etc.*).

The macrophage is the central component of the immune response system, and hence, modification of the phagocytic activity with perfluorocarbon emulsions can modulate the cellular and humoral immune reactions.

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