EFFECT OF LIVER DAMAGE ON THE BLOOD MUCOPROTEINS IN INFLAMMATORY PROCESSES

(UDC 616-002+616.72-002-031.13]-06:616.36-099-07:616.153.96)

F. S. Baranova

Laboratory of Physico-Biochemistry (Prof. A. A. Tustanovskii, Head),
Institute of Rheumatism (Prof. A. I. Nesterov, Member of the Academy of Medical Sciences, USSR, Director), AMN USSR, Moscow

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 8,
pp. 57-61, August, 1965
Original article submitted August 13, 1964

It is known that in acute rheumatic attacks and in other collagen diseases during their inflammatory periods, in myocardial infarction, and in infectious diseases, there is a substantial increase in the blood α-globulin level (haptoglobin, orosumucoid, and the Schultz α-1-antitrypsin [6]).

There are conflicting ideas as to the origin of the mucoproteins of the α-globulin fraction. According to some authors the level of the mucoproteins rises because of degradation of basic components in the tissue [4, 14, 16, 17]; according to others it is due to an increase in the hepatic synthesis of mucoproteins [2, 8, 9, 10, 18, 22].

The present investigation concerns the effect of carbon tetrachloride (CCl_4) on the mucoprotein level of the serum α-globulin fraction during localized inflammation and during experimental polyarthritis.

METHODS

The experiments were carried out on white male rats, 180-200 g in body weight. Inflammation was elicited by a single subcutaneous injection of turpentine in the right iliac region, the doses being adjusted to 0.12 ml per 100 g body weight. Experimental polyarthritis was produced by a single intradermal injection of 0.1 ml of a suspension made up of 3 mg of killed M. tuberculosis per ml of vaseline (Freund adjuvant) into the foot pad [20]. Polyarthritis developed on the 15th to 20th day after the stimulating dose. Animals were used that evidenced macroscopically apparent changes (the number of affected joints was taken into consideration). The presence of inflammatory changes in the joints in the acute exudative phase was confirmed by histological examination (in consultation with Prof. G. V. Orlovska).

The CCl_4 was administered both in experiments with local inflammation as well as with polyarthritis at the times indicated below in the following way: 0.25 ml per 100 g body weight as a 40% solution in sunflower seed oil, subcutaneously into the left iliac region.

In view of the fact that the mucoproteins under study belong by chemical composition to the sialomucoproteins, they were determined in the blood by the diphenylamine reaction [1, 5]. The results of the measurement were expressed in extinction units. In some of the experiments a parallel determination of sialic acid was made by the Warren [21] thiobarbiturate method in addition to the diphenylamine reaction. Similar results were obtained by both methods. In selected cases electrophoresis of the serum was carried out with quantitative estimation of the α-globulin fraction.

The study was made on 5 groups of rats, containing in all 35 animals; one group (7 rats) served as the control. In each time period 5 rats were sacrificed. Animals in the 1st group were given turpentine, the 2nd were given turpentine and CCl_4 simultaneously, the 3rd group received only CCl_4. Experimental polyarthritis was induced in the 4th group and 20 days after the Freund adjuvant was given they received CCl_4. The 5th group of rats (control) also was given polyarthritis and then they received only sunflower seed oil at the rate of 0.15 ml per 100 g body weight.

TABLE 1. The Change in Blood Serum of Rats Following the Administration of Turpentine and CCl_4 Individually and Simultaneously

Causative agent; statistical data	Normal	Days after start of experiment						
		1/2	1	1 1/2	2	3	4	6
Turpentine								
Number of experiments	7	5	5	5	5	5	5	5
Maximum and minimum values	0,2—0,121	0,19—0,100	0,31—0,150	0,45—0,210	0,3—0,150	0,3—0,200	0,3—0,200	0,27—0,190
$\bar{x} \pm m$	0,17±0,013	0,13±0,018	0,25±0,035	0,3±0,045	0,22±0,020	0,26±0,018	0,23±0,023	0,23±0,015
P		>0,1	>0,05	<0,05	>0,5	<0,01	<0,05	<0,02
Turpentine + CCl_4								
Number of experiments	7	5	5	5	5	5	5	5
Maximum and minimum values	0,2—0,121	0,15—0,100	0,17—0,120	0,16—0,110	0,15—0,100	0,25—0,170	0,27—0,210	0,25—0,140
$\bar{x} \pm m$	0,17±0,014	0,125±0,04	0,14±0,019	0,13±0,019	0,13±0,021	0,21±0,002	0,254±0,014	0,2±0,023
P		<0,05	>0,4	>0,5	>0,4	>0,5	<0,01	>0,9
CCl_4								
Number of experiments	7	5	5	5	5	5	5	5
Maximum and minimum values	0,2—0,121	0,18—0,100	0,15—0,090	0,16—0,095	0,2—0,100	0,3—0,200	0,3—0,210	0,28—0,200
$\bar{x} \pm m$	0,17±0,014	0,136±0,015	0,125±0,012	0,14±0,014	0,15±0,02	0,25±0,025	0,26±0,018	0,23±0,018
P		>0,5	<0,05	>0,6	>0,6	<0,02	<0,01	<0,01

TABLE 2. Change in Mucoprotein Content of Blood Serum in Experimental Polyarthritic Rats Produced by Administration of CCl_4 or of Sunflower Seed Oil on the 20th Day after Giving the Freund Adjuvant

Agent administered; statistical data	Normal	20th Day of expt.	Days from the start of experiment					
			1/2	1	1 1/2	2	3	4	6
CCl_4									
Number of experiments	5	5	5	5	5	5	5	5	5
Maximum and minimum values	0,18—0,130	0,3—0,230	0,17—0,120	0,18—0,130	0,17—0,120	0,18—0,150	0,2—0,120	0,25—0,180	0,3—0,210
$\bar{x}\pm m$	0,16±0,010	0,25±0,019	0,15±0,009	0,153±0,009	0,142±0,090	0,164±0,012	0,166±0,014	0,186±0,023	0,246±0,016
P	<0,01	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	>0,05	>0,3
Sunflower seed oil									
Number of experiments	5	5	5	5	5	5	5	5	5
Maximum and minimum values	0,18—0,13	0,3—0,23	0,27—0,22	0,29—0,200	0,3—0,210	0,27—0,230		0,28—0,240	0,3—0,220
$\bar{x}\pm m$	0,16±0,01	0,25±0,019	0,24±0,09	0,25±0,020	0,24±0,020	0,25±0,009	—	0,26±0,009	0,25±0,030
P		<0,01	>0,7	>0,7	>0,7	>0,7		0,7	0,7

Note: In the column "20th day of experiment" the magnitude of P was calculated in comparison with the normal. In the remaining cases, P was calculated in comparison with the value on the 20th day of experiment.

The data were treated statistically and the absolute values presented.

RESULTS

During inflammation elicited by local injection of turpentine, the serum mucoprotein was observed to increase starting at the 12th h, this having beenpreceded by a fall. The maximum elevation was noted after 36 h (Table 1). Simultaneous administration of CCl_4 and turpentine resulted in no increased mucoprotein level, but it did not result in any reversal of the inflammatory process. The administration of CCl_4 alone depressed the level of serum mucoproteins.

The changes in level of the α-globulin fraction, as detected electrophoretically, ran parallel to the change in mucoprotein level. It had been shown earlier [13] that the α-globulin level falls under the influence of CCl_4, and a progressive fall in the serum mucoprotein level is produced by repeated administration of CCl_4 [1, 2]. The authors consider that the fall in mucoprotein content is produced by damage to the liver parenchymal cells and concluded that the liver parenchyme is the site of mucoprotein synthesis.

We have noted that the regularity in relationships implied in this last conclusion does not invariably hold. The fall in level of mucoproteins during the 1st and 2nd day after a single CCl_4 and turpentine treatment is always replaced later (3rd-4th day) by a distinct elevation in the blood level of mucoproteins. The same situation may be observed when CCl_4 is administered alone.

It is known that CCl_4 is a specific liver poison and in 24 h after administration it produces dystrophic changes in the parenchymal cells with centrolobular necroses and the appearance of histidase and urocaninase in the blood serum [3]. Normal function of the parenchymal cells returns only 2-3 weeks following damage. However, the level of mucoproteins in the serum increases in 2 days after giving the poison. It is not very likely that the damaged cells of the liver parenchyme would be capable of increased mucoprotein synthesis at the height of the dystrophic process.

According to some information [15] there is a decreased content of RNA in the parenchymal cells of the injured liver, while the RNA content of the Kupfer cell cytoplasm increases. From this it is reasonable to assume that increased synthesis of the α-globulin mucoproteins results from the hepatic reticulo-endothelial system cells [11, 12] and that the fluctuations in the serum level of these proteins depend upon the functional state of this system.

Actually, the fall in the mucoproteins in the first 2 days after CCl_4 administration and the sharp increase on the 3rd day may be viewed as caused by primary injury to the hepatic reticulo-endothelial cells or secondary inhibition of their function (circulatory effect), followed by a rapid restoration of the mucoprotein by these cells which undergo characteristic hyperplasia [11], with consequent capacity for excess mucoprotein synthesis. Moreover, during acute CCl_4 injury in the first 2 h, "liver antigens" are found in the blood serum. These antigens reach a maximum concentration in 24 h [7]. It is known that the Kupfer cells of the liver have the ability to phagocytize the principal bulk of antigenic material appearing in the blood [19]. It is possible that a functional inhibition of the reticulo-endothelial system cells occurs and this results in a decrease in biosynthesis of the mucoproteins. Apparently, if CCl_4 and turpentine act simultaneously the inhibition or "blockade" is able to prevent the increased synthesis of mucoproteins. During the subsequent phase, hyperplasia and hyperfunction of the reticulo-endothelial cells takes place and this may be linked with increased synthesis of the mucoproteins.

Thus, the character of the changes in condition of the hepatic reticulo-endothelial cells appears to fit into the scheme which we have proposed: namely, that the synthesis of mucoproteins, the level of which increases during various pathological conditions, does not occur in the liver parenchymal cells but rather in the cells of the reticulo-endothelial system.

We obtained similar results in a study of the influence of CCl_4 on the serum mucoprotein in experimental polyarthritis (Table 2). On the 20th day after administering the Freund adjuvant, the mucoprotein content of the blood serum α-globulin fraction had increased to 160% of the control level. Increased synthesis of mucoproteins in experimental polyarthritis may be related to the reticulo-endothelial cell proliferation known to occur in this process [19]. When CCl_4 is administered on this same day it produces a fall in the mucoprotein level (see Table 2). There is no indication in the literature that CCl_4 has any direct suppressing action on the process of connective tissue destruction during inflammatory processes.

Thus, the fall in level of mucoproteins under the influence of CCl_4 under the conditions of experimental polyarthritis and local inflammation, apparently, is associated with hypofunction of the hepatic reticulo-endothelial system cells. Increase in the level of mucoproteins in the later days after CCl_4 administration is to be explained in both cases by the restored function of the cells in this system and by their hyperplasia.

It may be postulated that excessive synthesis of the α-globulin fraction mucoproteins is mediated by the functional state and progressive hyperplasia of the cells in the hepatic reticulo-endothelial system in various other pathological conditions.

SUMMARY

The injection of CCl_4 to intact rats causes a decrease in the first 2 days and a marked increase on the 3rd-4th day in the mucoprotein content of the blood. Fluctuations in the mucoprotein content of the blood, analogous in character and terms, are observed during the action of CCl_4 in local (caused by turpentine) and diffuse (acute exudative polyarthritis) inflammatory processes.

These facts corroborate the suggestion that synthesis of mucoproteins of α-globulin fraction of the blood serum takes place not in the liver parenchyme cells but in the cells of the reticulo-endothelial system and that fluctuations in the mucoprotein level in various inflammatory processes are directly related to the functional conditions of these cells.

LITERATURE CITED

1. É. G. Larskii, Labor. delo, 4 (1957) p. 13.
2. É. G. Larskii, Vopr. med khimii, 5 (1961) p. 505.
3. S. R. Mardashev and V. A. Burobin, Vopr. med. khimii, 3 (1962) p. 320.
4. A. J. Anderson, C. H. Lack, and S. Y. Ali, Nature 197 (1963) p. 707.
5. W. Ayala, L. V. Moore, and E. L. Hess, J. clin. Invest., 30 (1941) p. 781.
6. S. Belfrage, Acta med. scand., 173, 395 (1963).
7. E. Espinosa and I. Insunza, Proc. Soc. exp. Biol. (New York) 111 (1962) p. 174.
8. E. Greenspan, Advanc. intern. Med., 7 (1955) p. 101.
9. L. L. Miller and W. F. Bale, J. exp. Med., 99 (1954) p. 125.
10. L. L. Miller, C. G. Bly, and W. F. Bale, Ibid., p. 133.

11. F. Moyson, Rev. belge Path., 25 (1956) p. 190.
12. F. Moyson and A. Steens-Lievens, Biochim. biophys. Acta, 21 (1956) p. 500.
13. O. W. Neuhaus, H. F. Balegno, and A. T. Milauskas, Am. J. Physiol., 202 (1962) p. 257.
14. C. L. Pirani and H. R. Catchpole, Arch. Path., 51 (1951) p. 597.
15. H. Popper (1951) Citation by A. Fisher. Physiology and experimental pathology of the liver [in Russian] Budapest (1961) p. 15.
16. L. Robert, P. Mombelloni, and P. Crosti, Proc. Soc. exp. Biol. (New York) 107 (1961) p. 499.
17. F. B. Seibert et al., J. clin. Invest., 26 (1947) p. 90.
18. M. R. Shetlar et al., Cancer Res., 9 (1949) p. 515.
19. J. F. Snell, Ann. New York Acad. Sci., 88, 1 (1960) p. 56.
20. J. R. Ward and R. S. Jones, Arthr. and Rheum., 5 (1962) p. 557.
21. L. Warren, J. biol. chem., 234 (1959) p. 1971.
22. I. Werner, Acta physiol. scand, 19 (1949) p. 27.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
