disease. We know that the quantity of fibronectin is not only subject to individual variations, but also differs in different age groups (in children (a particularly vulnerable contingent as regards meaningococcal infection) the fibronectin level is significantly lower than in adults [5]). On the other hand, the results point to a common type of fibronectin receptor in miningococci of different strains. This receptor is evidently one of the common meningococcal antigens, interest in which is explained by the search for preparations capable of exerting a protective effect against meningococcal infection of whatever specificity. Thus elucidation of the concrete mechanism of ligand-receptor interactions in the chain of processes leading to adhesion of the menigococcus to epithelial cells requires further study.

LITERATURE CITED

1. E. K. Fan'kovskaya, N. N. Kostyukova, and I. I. Kruman, Zh. Mikrobiol., No. 9, 92 (1975).
2. D. Brener and I. M. DeVoe, Curr. Microbiol., 8, 57 (1983).
3. E. Engvall and E. Ruoslahti, J. Cancer, 20, 1 (1977).
4. B.-E. Kristiansen, B. Sorensen, T. Simonsen, et al., J. Infect. Dis., 150, 389 (1984).
5. M. H. McCafferty, M. Lepow, T. M. Saba, et al., Pediat. Res., 17, 482 (1983).
6. Z. A. McGee, C. H. Street, C. L. Chappel, et al., Infect. Immun., 24, 194 (1979).
7. W. A. Simpson, H. Courtney, and E. H. Beachey, Microbiology, D. Schlesinger (ed.), Washington (1982), p. 346-347.
8. W. A. Simpson and E. H. Beachey, Infect. Immun., 39, 275 (1983).
9. W. A. Simpson, D. L. Hasty, and E. H. Beachey, Infect. Immun., 48, 318 (1985).
10. L. van de Water, A. T. Destree, and R. O. Hynes, Science, 220, 201 (1983).
11. D. E. Woods, D. C. Straus, W. G. Johanson, et al., J. Infect. Dis., 143, 784 (1981).

EFFECT OF PLATINUM PREPARATION ON PHAGOCYTIC ACTIVITY OF MOUSE PERITONEAL EXUDATE MACROPHAGES

E. V. Brin and B. S. Uteshev

UDC 612.112.3.017.1.014.46:[615.277.3:546.92

KEY WORDS: peritoneal macrophages, chemiluminescence, coordination compounds of platinum

A wide range of effect of cells is involved in the realization of antitumor immunity: natural killer cells, cytotoxic T lymphocytes, and macrophages. The last of these are the most interesting. Macrophages can cause lysis of various types of tumor cells, without damaging normal cells of the same histogenesis [4, 6, 8]. Normal "unarmed," unactivated macrophages can interact with tumor cells at the stage of their appearance and during the initial stage of their development. Cytostatics used in the chemotherapy of neoplasms influence the immune system of the host and, in particular, damage the mononuclear system. The effect of various classes of cytostatics on function of the macrophagal component of immunity has been studied in sufficient depth [5]. However, data on the character of the effect of a new class of antitumor compounds, namely coordination compounds of platinum, on macrophages could not be found in the accessible literature. The aim of this investigation was to determine the action of platinum preparations on phagocytic activity of peritoneal exudate macrophages.

EXPERIMENTAL METHOD

Oxoplatinum (cis-dichlorodiamino-trans-dihydroxoplatinum IV, produced by "Lachema," Czechoslovakia) and cycloplatam [cyclopeptidylamino-S-malatoplatinum (II) amine], produced in the USSR, were generously provided for the study by Professor A. B. Syrkin M.D. (All-Union Oncologic Scientific Center, Academy of Medical Scientific Center, Academy of Medical Sciences of the USSR). The preparations were made up when required in 5% glucose solution to give doses of between 0.01 and 1.0 MAD (LD_{10}) in experiments in vivo, and in complete medium RPMI-1640 to concentrations of 0.09-90.0, or 0.46-46.0 μg/ml for oxoplatinum and cycloplatam,

N. I. Pirogov Second Moscow Medical Institute.

(Presented by Academician of the Academy of Sciences of the USSR Yu. A. Vladimirov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, pp. 513-515, November, 1990. Original article submitted December 28, 1989.

TABLE 1. Effect of Oxoplatinum on Phagocytic Activity of Peritoneal Macrophages

Dose, mg/kg, fraction of MAD	in vitro	Nonimmune mice (I_{CL}) in vivo					Immune mice (I_{CL})				
days of injection		+1 day	+2 day	+3 day	+4 day	+5 day	+1 day	+2 day	+3 day	+4 day	+5 day
Control	1,69	3,8	2,95	2,77	2,1	1,42	1,3	5,19	2,55	4,7	1,14
1.0 MAD = 90.0 mg/kg	1,79	2,3	2,88	—	1,5	8,7	106,9	1,76	2,75	5,9	3,0
$\frac{1}{2}$ MAD = 45.0 mg/kg	1,88	1,1	—	2,43	1,9	4,5	110,0	2,05	4,54	8,0	2,9
$\frac{1}{4}$ MAD = 22.5 mg/kg	2,62	2,4	2,66	6,55	2,96	8,3	21,4	7,75	2,36	5,0	5,9
$\frac{1}{8}$ MAD = 11.25 mg/kg	1,65	1,6	1,8	2,15	2,4	1,9	16,9	9,93	4,28	4,6	4,1

respectively, for experiments in vitro. To study dose–effect dependence the preparations were injected intraperitoneally in various doses into C57Bl/6 mice in a volume of 0.5 ml simultaneously with or without antigenic stimulation, at different times relative to the day of the experiment (over a period of 1-5 days of dose–effect dependence). Phagocytic activity of the mouse peritoneal exudate macrophages was evaluated by the chemiluminescence method [9]. The level of chemiluminescence reached a peak during zymosan-induced phagocytosis. The results were expressed per $1 \cdot 10^5$ macrophages. For this purpose films of peritoneal exudate cells were prepared and stained with a dye containing 31% azure and 15% eosin, and the number of macrophages in each test sample was counted. The number of pulses emitted by $1 \cdot 10^5$ macrophages (M) was calculated by the formula:

$$M = \frac{m \cdot 100\,\%}{H \cdot n\,\%}, \qquad (1)$$

where H is the number of millions of peritoneal exudate cells in 1 ml of the test suspension (determined in a Goryaev counting chamber), n% denotes the percentage of macrophages in the test sample (as shown by staining of the films), and m the number of pulses in the given sample at the peak of chemiluminescence.

Indices of chemiluminescence for the control and experimental sample were calculated as the ratio of M_2 in the samples with zymosan-induced phagocytosis and M_1 for spontaneous chemiluminescence (SCL).

EXPERIMENTAL RESULTS

It was found in the course of the experiments that addition of the platinum preparations directly to the tubes for counting, during recording of chemiluminescence in vitro, that there was a very small increase in release of the hydroxyl radical (OH^-), the superoxide anion (O^{2-}), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), indirect evidence of stimulation of phagocytic activity of the peritoneal macrophages by the platinum preparations in vitro. For cycloplatam, for instance, the maximal increase in formation of active oxygen metabolites was observed in a dose equal to 0.5 MPD (LD_{10}) = 23 mg/kg, and the index of chemiluminescence was 3.2 compared with 2.28 in the control, whereas addition of oxoplatinum in a dose of 0.25 MAD to the suspension of peritoneal macrophages caused an increase in the index of chemiluminescence from 1.69 in the control samples to 2.62 in the experiment (Tables 1 and 2). A similar picture of stimulation of secretion of active oxygen metabolites was observed [7] in a study of the effect of cisplatin, a precursor of the preparations which we studied [11]. It is interesting to note that according to data obtained by the same workers, low doses of cisplatin (5 mg/kg = 0.8 MAD) had a stronger stimulating effect than high doses 10 mg/kg = 1.25 MAD). Other workers also have found a similar relationship [1], based on the results of their own investigations of the effect of "platidiam," which also belongs to the class of platinum coordination compounds, on the percentage of active phagocytes and on their ingestive capacity. The authors described an increase in the ingestive capacity of the phagocytes by more than 1.5 times with the use of low doses of platidiam, whereas the use of high doses of the cytostatic was accompanied by disturbance of mononuclear phagocytic function.

Inconsistent and quite contradictory results were obtained during the subsequent investigation of the effect of oxoplatinum and cytoplam on phagocytic function of peritoneal macrophages in vivo (after injection of the preparations intraperitoneally into mice). As Tables 1 and 2 show, injection of oxyplatinum and cytoplatam into nonimmune mice caused suppression of of phagocytosis (on the 1st day after injection of cytoplatam in all doses, on the 1st and 2nd days after injection of oxoplatinum in all doses, with a stimulating effect of both preparations observed on the following days.

TABLE 2. Effect of Cytoplatam, Administered at Different Times of Immune Response, on Phagocytic Function of Mouse Peritoneal Macrophages (index of chemiluminescence I_{CL})

Dose, mg/kg, fraction of MAD / Days of injection	in vitro	In vivo									
		nonimmune mice					immune mice				
		day + 1	+2 day	+3 day	+4 day	+5 day	+1 day	+2 day	+3 day	+4 day	+5 day
Control	2,28± 0,16	3,8± 0,73	1,8± 0,26	1,9± 0,04	2,1± 0,12	1,55± 0,03	2,55± 0,3	2,48± 0,17	3,0± 0,13	2,54± 0,21	1,89± 0,14
46.0 mg/kg 1.0 MAD	2,36± 0,13	2,7± 0,21	10,47± 1,96	—	3,8± 0,71	2,9± 0,42	407,8± 32,71	3,52± 0,67	9,03± 2,22	2,7± 0,32	2,64± 0,7
23.0 mg/kg $\frac{1}{2}$ MAD	3,2± 0,22	2,7± 0,06	2,4± 0,11	2,8± 0,45	2,6± 0,25	2,7± 0,4	9,1± 0,98	2,93± 0,24	4,4± 0,53	5,7± 1,13	—
11.5 mg/kg $\frac{1}{4}$ MAD	2,4± 0,13	—	2,88± 0,1	5,13± 0,6	4,0± 0,27	1,4± 0,07	18,8± 3,8	2,23± 0,12	2,14± 0,12	7,1± 1,8	—
5.75 mg/kg $\frac{1}{8}$ MAD	2,33± 0,33	2,9± 0,62	2,85± 0,22	1,34± 0,05	2,95± 0,33	—	1,9± 0,5	2,03± 0,35	2,56± 0,31	3,1± 0,13	1,38± 0,16

However, injection of oxoplatinum and cytoplatam in the same doses and at the same times, together with antigenic stimulation, gave the opposite effect. On the 1st day after injection both preparations caused a dose-dependent increase in the index of chemiluminescence by two or more orders of magnitude (index of chemiluminescence I_{CL} for oxoplatinum in a dose of 1.0 MAD ws 106.9, for cycloplatam in a dose of 1.0 MAD it was 407.0, whereas in the control it was 1.3-2.5). On the following days, after injection of the preparations into immune mice, a stimulating effect on phagocytic activity of the peritoneal macrophages was clearly observed with all doses, but it was weaker (Tables 1 and 2).

In our view, when this phenomenon is explained, the fact must not be forgotten that peritoneal macrophages are heterogeneous, and a response to intraperitoneal injection of an alloantigen is inevitable, in the form of redistribution of subpopulations of peritoneal macrophages in favor of the "inflammatory" kind, at the expense of the "resident" type. The appearance of immature resident macrophages cannot be ruled out [2], for these likewise are characterized by higher peroxidase activity.

However, it is possible that when the reaction is carried out in this way, what was recorded was the fact that particles which could have been (and evidently were) not only zymosan granules, but also heterologous sheep's erythrocytes, were taken up and ingested by the peritoneal macrophages. This conclusion is supported by the results of a study by Kh. M. Isina, working in I. Ya. Uchitel's laboratory [3], to show that on the 1st day after immunization maximal uptake, ingestion, and destruction of heterologous erythrocytes by macrophages takes place, and this is followed (until the 48th hour) by stabilization of the process. We therefore suggest that the 1st day of administration cannot be regarded as a time of initiation of the stimulating effect of oxoplatinum and cyclopatam on phagocytic activity of peritoneal macrophages, whereas the results obtained on subsequent days provide reliable confirmation of this phenomenon.

LITERATURE CITED

1. N. V. Vasil'ev, V. M. Plotnikov, and A. G. Potapov, Byull. Sib. Otdel. Akad. Med. Nauk SSSR, No. 6, 139 (1987).
2. V. M. Zemskov, V. I. Pantin, and S. V. Rodionov, Immunologiya, No. 1, 31 (1986).
3. Kh. M. Isina and M. Ya. Korn, Tsitologiya, No. 5, 26 (1975).
4. J. B. Hibbs, L. H. Lambert, and J. S. Remington, Proc. Soc. Exp. Biol. (New York), 139, No. 3, 1049 (1972).
5. A. Mantovani, Adv. Pharmacol. Chemother., 19, 35 (1982).
6. W. P. Reed and Z. J. Lucas, J. Immunol., 115, No. 2, 395 (1975).
7. A. Sodhi and P. Gupta, Int. J. Immunopharmacol., 8, No. 7, 709 (1986).
8. C. C. Stewart, C. Adles, and J. B. Hibbs, The Reticuloendothelial System in Health and Disease: Immunologic and Pathologic Aspects, New York (1976), pp. 423-433.
9. T. Tatsuhito, N. Ueno, T. Matsumoto, et al., Clin. Immunol. Immunopath., 26, No. 6, 66 (1983).