EFFECTS OF GOLD AND PLATINUM ON NECROTIZING FACTOR, SKIN SENSITIZING ANTIBODY, AND COMPLEMENT

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Clinically interesting substances, such as necrotizing factor in rheumatoid arthritis, skin sensitizing antibody and complement in allergic diseases were easily inactivated by heating or even keeping them in room temperature. And there are many reports that heavy metal ions inhibit the activity of physico-chemically labile substances such as some kind of enzyme. On the other hand, gold compound was proved to have an antirheumatic effect and probably a therapeutic effect in some allergic disease. Colloidal platinum and palladium were tried to apply in treatment of rheumatism in Japan.

In this experiment, inhibitory effects of metal compounds including gold and platinum on the necrotizing factor, skin sensitizing antibody and complement were studied.

EXPERIMENTS AND RESULTS

1) *Effects on Necrotizing Factor*

Method: Metal compounds given in Table 1 were dissolved in distilled water and neutralized with NaOH. Serum was obtained from fresh clotted blood from active rheumatoid patients. A 0.475 ml of the serum was mixed with 0.025 ml of the metal compounds solution and incubated for 2 hours at 37°C. In the control group 0.025 ml of water was added in place of the metal solutions. Immediately after obtaining the blood from patients, the blood was covered with liquid paraffin, in order to avoid the contact with air. The whole procedures were carried out also in the solution covered with liquid paraffin.

A 0.1 ml of the serum incubated with metal compounds was injected intradermally into guinea pigs, which weighed about 300 g. Necrotizing activity was recorded 2 hours after the injection, judging from the intensity of the inflammation as described in previous paper (1). Only the sera which caused a strong inflammation (++) were used in this experiment.

Results: At least three samples each from two patients were examined and the results are summarized in Table 1. As shown in Table 1, ionized gold and platinum inhibited the necrotizing activity markedly at a concentration over 2×10^{-4} M. However,

TABLE 1. Effects of metal compounds on necrotizing activity.

Metal	Chemical formula	Final concentration			
		10^{-3} M	4×10^{-4} M	2×10^{-4} M	10^{-4} M
Pt	$\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$		—	+	++~#
Au	$\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$	±	±~++	+~++	
	Aurothioglucose			##	##
Pd	PdCl_2	±~+			
Hg	HgCl_2	±*	##*	##	
Cu	CuSO_4			##	
Ag	AgCl	±~++	##		
Fe	FeCl_3	++~	##		
Zn	ZnSO_4	~++	++		
Control			##		

++ strong, — medium, + weak, - no necrotizing activity.

* HgCl_2 alone caused a similar inflammation.

nonionized gold and other examined metals did not prove to have such an effect by this method.

2) Effects on Skin Sensitizing Antibody

Method: Serum was obtained aseptically from clotted blood from asthmatic patients. A 0.9 ml of the obtained serum was mixed with 0.1 ml of the solution contained metal compounds, which was prepared with the same method described in the above experiment. In control sample 0.1 ml of water was added instead of the metal solutions. The mixture was incubated for one hour at 37°C . Then the Prausnitz-Küstner Test was carried out with the serum and House Dust Extract. Namely, 0.1 ml of the serum injected intracutaneously to forearm skin of healthy persons, who show a negative skin reaction with $1,000\times$ House Dust Extract. Four hours after the injection of the serum 0.05 ml of $1,000\times$ House Dust Extract was injected into the site, in which the serum had been administered. Erythema appeared in the site was measured 15 minutes after the second injection.

Results: Mean diameter of the erythema is shown in Table 2. Mean diameter in the control group was larger than that in the platinum group in average. However, an apparently significant difference between them was not proved by this method.

3) Effects on Immune Hemolysis

Method: Fresh guinea pig's serum was diluted 1 to 20 in $1/10$ M phosphate buffered saline. A 0.1 ml of saline solution of metal compounds at a concentration of 10^{-3} M was added to the diluted serum and incubated for 2 hours at 37°C . After cooling the mixture, 0.25 ml of saline containing 8 units of rabbit hemolytic amboceptor, and 0.25 ml of a fresh 2% sheep erythrocytes suspension in saline were added to the mixture. During this procedure test tubes were kept in an ice water bath. Then whole mixture was incubated further for 6 minutes at 37°C . The mixture was cooled and centrifuged.

TABLE 2. Effects of ionized gold and platinum on skin sensitizing antibody (by means of Prausnitz-Küstner reaction).

No.	Control	Mean diameter of erythema (cm)			
		Gold		Platinum	
		10^{-3} M	5×10^{-4} M	10^{-3} M	5×10^{-4} M
1	28			8	
2	25			7	
3	23			7	
4	27			14	
5	17	17		16	
6	20	14		14	
7	35	21		21	
8	24	43		28	
9	24	29		32	
10	19	16		20	
11	18	19		12	
12	16		13		12
13	25		20		15
14	33		25		30
15	27		28		18

Sera from asthmatic patients were incubated with metal ions at a final concentration given in Table.

TABLE 3. Comparative anticomplementary effect of metal compounds at final concentration of 10^{-4} M.

Percent of inhibition	Metal	Chemical formula
100%	Pt	$H_2PtCl_6 \cdot 6H_2O$
	Au	$HAuCl_4 \cdot 4H_2O$
	Hg	$HgCl_2$
over 90%	Cu	$CuSO_4$
over 70%	Pb	PbO_2
over 50%	Zn	$ZnSO_4$
over 10%	Mn	$MnCl_2$
under 10% and none	Ag	$AgNO_3$
	Sn	$SnCl_2$
	Ni	$Ni(HCOO)_2$
	Co	$CoCl_2 \cdot 6H_2O$
	Fe	$FeCl_3$
	Mg	$MgCl_2$
	Au	Aurothioglucose
	Hg	PCMB
	Cu	Glycine-Cu

Percentage of inhibition of lysis by the added drugs was calculated by measuring the color of supernatant of the mixture with spectrophotometer with green filter (530 $m\mu$).

Results: Time needed for complete hemolysis varies depending on the complement titer of guinea pig's serum. The results given in Table 3 refer to the antihemolytic effect of metal compounds in the experiment using a guinea pig's serum, which gave a complete hemolysis within 3-4 minutes. Table 3 indicates that anticomplementary effect of gold and platinum ions was the strongest among the tested metal compounds. However aurothioglucose gave a negative result.

When 0.25 ml of saline with 8 units of amboceptor was incubated with 0.1 ml of the metal solution and after that fresh guinea pig's serum and sheep erythrocytes were added to the mixture, no marked inhibition was observed. Therefore, the anti-hemolytic effect of metal compounds was considered mostly due to their actions on complement.

DISCUSSION

Necrotizing factor in the sera from rheumatic patients is a labile inflammation factor and causes a severe necrosis of guinea pig's skin by intradermal injection. Chemical, biological and immunological properties of the necrotizing factor were investigated in our previous studies (1, 2). In the present experiment ionized gold and platinum inhibited markedly the activity of this labile inflammation factor.

Complement is also a labile substance and was reported to be inactivated by metal

compounds such as Zn^{++} (4) and Cu-chlorophyllin (5). In this study ionized gold and platinum were found to have the strongest anticomplementary effect among the tested metal ions. But a specific fall in complement titer of the serum from rheumatoid patients who were treated with gold compounds was not evident (8). Gold sodium thiosulfate was reported to fail in altering demonstrably the magnitude of the Arthus phenomenon in rabbit (9).

Cooke reported that skin sensitizing antibody in ragweed allergy was labile especially in acid solution (3). From this study ionized platinum seemed to have a weak inhibitory action on skin sensitizing antibody. Further, in our unpublished experiment it was found that fresh goat serum, which also causes a severe inflammation of guinea pig's skin, was thermolabile and its biological activity was inhibited markedly by a small amount of gold and platinum ions.

These inhibitions of biologically active substances by gold and platinum might be explained partly by their actions on SH radical. Gerber reported the action of gold compound on SH radical of proteins (6). Gold concentration in serum obtained in gold treatment was reported to be about 5×10^{-5} M. But tissue concentration of gold was reported much higher (7). Therefore inhibitory action of gold and platinum on certain labile and biologically active substances may have some connection to their mode of action in rheumatic and allergic diseases.

SUMMARY

Necrotizing factor in the serum from rheumatoid patients was inhibited markedly by ionized gold and platinum at a concentration of over 2×10^{-4} M, but not inhibited so strongly by other metal compounds tested. Ionized platinum seemed to have a weak inhibitory action on skin sensitizing antibody, but significant result was not obtained by this experiment and is open to question. Anticomplementary effect of ionized gold and platinum was found to be the strongest among the metal ions.

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