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Cooperation of chloroquine and blood platelets in inhibition of polymorphonuclear leukocyte chemiluminescence☆

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Abstract

Effect of activated blood platelets and chloroquine on concentration of reactive oxygen species produced by polymorphonuclear leukocytes (PMNL) stimulated with Ca^{2+}-ionophore A23187 was investigated. Oxygen metabolites localized outside PMNL were visualized by isoluminol enhanced chemiluminescence, whereas chemiluminescence, enhanced with luminol and measured in the presence of the extracellular scavengers superoxide dismutase and catalase, was used for the detection of radicals originated intracellularly. Significant reduction of chemiluminescence was observed in the presence of platelets (added to PMNL in the physiological cell ratio 50:1) and of chloroquine (10 and 100 μmol/L). Although chloroquine decreased effectively both the extra- as well as the intracellular part of the chemiluminescence signal, the activity of platelets occurred largely outside PMNL. Serotonin liberated from platelets by A23187 appeared to be involved in inhibition of chemiluminescence; its concentrations achieved in platelet supernatants were found to be sufficient for elimination of PMNL-derived oxygen metabolites. The presented results indicated that chloroquine and blood platelets cooperate in inhibition of chemiluminescence because their common effect was found to be much more extensive than reduction induced by these inhibitors separately. Therefore, for accurate prediction of drug effect in the whole organism, the use of multicellular test systems seems to be pertinent.

Keywords: Human blood platelets; Polymorphonuclear leukocytes; Chloroquine; Chemiluminescence; Ca^{2+}-ionophore A23187; Serotonin

1. Introduction

Activated polymorphonuclear leukocytes (PMNL) produce a great number of oxygen metabolites (e.g. superoxide anion, hydrogen peroxide, hydroxyl radical, singlet oxygen) that are of utmost importance in the protection of the organism against infection [1,2]. However, mechanisms removing these highly reactive substances are equally important because they can protect tissues in the proximity of activated PMNL.

Besides numerous extracellular scavengers and antioxidants, the protective function is also fulfilled by some cells coexisting with PMNL at the site of inflammation. In circulation, blood platelets, accumulated and activated simultaneously with PMNL, contain and liberate substances that can eliminate or decrease synthesis of oxygen metabolites [3–6]. As we found previously, the ability of platelets to inhibit PMNL chemiluminescence is connected with their activation because resting platelets displayed a rather potentiating effect [7].

Under pathological conditions, reduced concentration of reactive oxygen species is achieved by drugs that either suppress PMNL functions or quench existing oxygen metabolites in extracellular space. The antimalarial drug chloroquine possesses one of these properties [8–11], and it is successfully used in the therapy of autoimmune diseases accompanied with inflammation —particularly lupus erythematosus and rheumatoid arthritis [12–15].

Because both blood platelets as well as chloroquine effectively decreased PMNL activity, it was of interest to investigate whether this inhibitory effect would be potentiated by their simultaneous application. Moreover, the pos-

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Abbreviations: CAT, catalase; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; PMNL, polymorphonuclear leukocytes; PRP, platelet rich plasma; SOD, superoxide dismutase.

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sible role of platelet-liberated serotonin in reduction of PMNL chemiluminescence was tested.

2. Materials and methods

2.1. Materials

Calcium ionophore A23187, dextran (average mol. wt. 464,000), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), isoluminol (6-amino-2,3-dihydro-1,4 phthalazinedione), and superoxide dismutase (SOD; from bovine erythrocytes) were from Sigma-Aldrich Chemie (Deisenhofen, Germany); horseradish peroxidase (HRP) and catalase (CAT) (from beef liver) from Merck (Darmstadt, Germany); lymphoprep (density 1.077 g/mL) from Nycomed Pharma AS (Oslo, Norway); serotonin (5-hydroxytryptamine creatinine sulphate) from Koch Light (Colbrook Bucks, UK); and chloroquine phosphate from ACO (Molndal, Sweden). All other chemicals of analytical grade were from available commercial sources.

2.2. Isolation of blood platelets

Isolation was performed as described previously [16]. Briefly, fresh blood was obtained at the blood bank by venepuncture from healthy male donors (20–50 years) who had not received any medication for at least 7 days. Blood samples (36 mL) were anticoagulated with 3.8% trisodium citrate (4 mL) and centrifuged at 260 $\times$ *g* for 15 min. Platelet rich plasma (PRP) was removed, mixed with a solution containing 4.5% citric acid and 6.6% glucose (50 μL/mL PRP), and centrifuged at 1070 $\times$ *g* for 10 min. Platelets were resuspended in an equal volume of Tyrode's solution (136.9 mmol/L NaCl, 2.7 mmol/L KCl, 11.9 mmol/L $NaHCO_3$, 0.4 mmol/L $NaH_2PO_4.2H_2O$, 1 mmol/L $MgCl_2.6\ H_2O$, and 5.6 mmol/L glucose) containing 5.4 mmol/l EDTA, pH 6.5. After 10-min stabilization, the suspension was centrifuged for 6 min at 1070 $\times$ *g* and platelets were resuspended in the same buffer without EDTA (pH 7.4) to obtain 2×10^5 (chemiluminescence) or 5×10^5 (serotonin) platelets per 1 μL.

2.3. Isolation of PMNL [17]

After removal of PRP (see Isolation of Blood Platelets), the blood volume was reconstituted with 0.9% NaCl. Then a 3% dextran solution (1% final concentration) was added, and blood was allowed to sediment (1 $\times$ *g*) for 25 min at 22°. The leukocyte/dextran mixture was centrifuged at 500 $\times$ *g* for 10 min, and the pellet was resuspended in phosphate-buffered saline (PBS: 137 mmol/L NaCl, 2.7 mmol/L KCl, 8.1 mmol/L Na_2HPO_4, and 1.5 mmol/L KH_2PO_4, pH 7.4). Leukocyte suspension (3 mL) was layered on lymphoprep (3 mL) and centrifuged 30 min at 500 $\times$ *g*. Contaminating red blood cells were removed by hypotonic lysis (3 mL of icy deionized water followed after 45 s by 3 mL of 1.8% NaCl and 4 mL PBS). After centrifugation (500 $\times$ *g*, 10 min) PMNL was washed in PBS, stained with Turck solution, and counted under light microscope. Final suspension (in PBS) contained 10^4 PMNL per 1 μL.

2.4. Chemiluminescence assay

Chemiluminescence of PMNL was measured using two systems. In System I, chemiluminescence was amplified with isoluminol and measured in the presence of HRP. Addition of peroxidase ensured that detection of extracellularly localized oxygen metabolites was complete and not limited by insufficient liberation of myeloperoxidase from azurophil granules of PMNL [18]. The reaction mixture consisted of 200 μL PMNL (2×10^6), 155 μL PBS, 50 μL 18 mmol/L $CaCl_2$, 25 μL 1 mmol/L $MgCl_2$, 20 μL 400 U/mL HRP, and 500 μL platelets (10^8) or Tyrode's solution. Only one neutrophil:platelet ratio (1:50) was tested because it reflects the physiological cell proportion, and the effect of platelets was found to rise proportionally with their number [19,20] (our unpublished data). After 1 min incubation at 37° and stirring at 1000 rpm, 20 μL of chloroquine (1, 10, and 100 μmol/L final concentration) and 2 min later 20 μL of isoluminol (5 μmol/L final concentration) were added. Samples were incubated for further 3 min, stimulated with 10 μL of A23187 (1 μmol/L final concentration), and chemiluminescence was measured in a lumiaggregometer, model 500 (Chrono-log, Corp.). Serotonin (20 μL) was added 30 s before A23187.

In System II, SOD (100 U/mL) with CAT (2000 U/mL) was added instead of peroxidase, and isoluminol was replaced by luminol. The chemiluminescence signal was recorded by a dual pen recorder (Chrono-log, Corp.) at an appropriate sensitivity setting and chart speed 10 mm/min. The presented results (mV) refer to the peak value recorded.

2.5. Serotonin liberation

A slight modification of the method described by Nosal *et al.* [16] was used for determination of platelet serotonin. After 6 min pre-incubation at 37°, isolated platelets (1100 μL, 5×10^5 cells/μL) were stimulated by addition of 50 μL of Ca^{2+}-ionophore A23187 (final concentration 1 μmol/L). Serotonin liberation was stopped after 5 min by cooling the samples to 4° and by immediate centrifugation at 14000 $\times$ *g* and 4° for 2 min. Supernatant was decanted to another tube, sediment was resuspended in 1100 μL of 0.02 N HCl, and subsequently sonicated for 20 min at 22°. For determination, 400 μL of supernatant (or sediment), 2600 μL of 0.02 N HCl, 200 μL of 10% w/w $ZnSO_4$, and 100 μL of 1N NaOH were mixed and centrifuged for 10 min at 1670 $\times$ *g*. The native fluorescence of serotonin was measured in Perkin Elmer Fluorescence Spectrometer Model 203 at 300 and 332 nm excitation and emission wavelength, respectively.

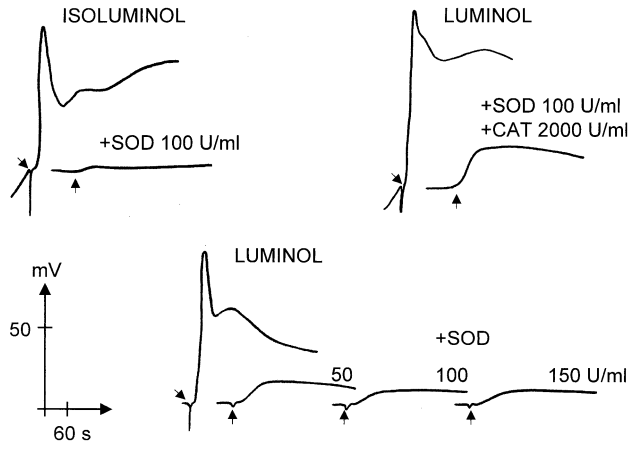

Fig. 1. Effect of extracellular scavengers, SOD and CAT, on PMNL chemiluminescence stimulated with A23187. Chemiluminescence, enhanced with isoluminol or with luminol, was measured in the presence of HRP (8 U/mL). SOD and CAT were applied 5 min before chemiluminescence initiation. Arrows indicate addition of stimulus. Curves displayed are representative of five donors.

2.6. Data analysis

Commercially available computer statistic programs were used for calculations of means and S.E.M. Statistical significance of differences between means was established by Student's *t*-test and $P \leq 0.05$ was taken as significant.

3. Results

To investigate the effect of stimulated platelets on activated PMNL, Ca^{2+}-ionophore A23187 was used because it is capable of stimulating both types of cells. Formation of oxygen metabolites in PMNL was measured by the chemiluminescence method, and the chemiluminescence originated inside PMNL was selected by means of extracellular scavengers —SOD and CAT. Chemiluminescence enhanced with isoluminol (assay System I) was completely blocked with 100 U/mL of SOD (Fig. 1), indicating that this chemiluminescence signal was associated predominantly with extracellularly localized oxygen metabolites, namely with superoxide anion. On the other hand, the chemiluminescence enhanced with luminol was decreased only partially in the presence of SOD, and neither simultaneous application of SOD with CAT nor increased SOD concentration resulted in complete inhibition of the chemiluminescence signal. It indicated that the luminol enhanced chemiluminescence measured in the presence of SOD and CAT (assay System II) detected reactive oxygen species unavailable to the effect of extracellular scavengers, i.e. radicals localized intracellularly.

The extracellular effect of chloroquine and blood platelets on stimulated PMNL, detected by means of isoluminol enhanced chemiluminescence, is presented in Fig. 2. Chloroquine (Panel A), in the concentration of 10 and 100 μmol/L, significantly decreased PMNL chemiluminescence from 85.63 ± 10.27 mV (control) to 57.48 ± 8.17 and 11.84 ± 1.37 mV, respectively. Platelets (Panel B) were found to be more effective because their addition to PMNL in the physiological cell ratio 50:1 declined chemiluminescence to 3.96 ± 1.23 mV. The simultaneous application of platelets with 10 or 100 μmol/L chloroquine (Panel B) resulted in further reduction of chemiluminescence to 2.06 ± 0.45 or 0.45 ± 0.08 mV, respectively.

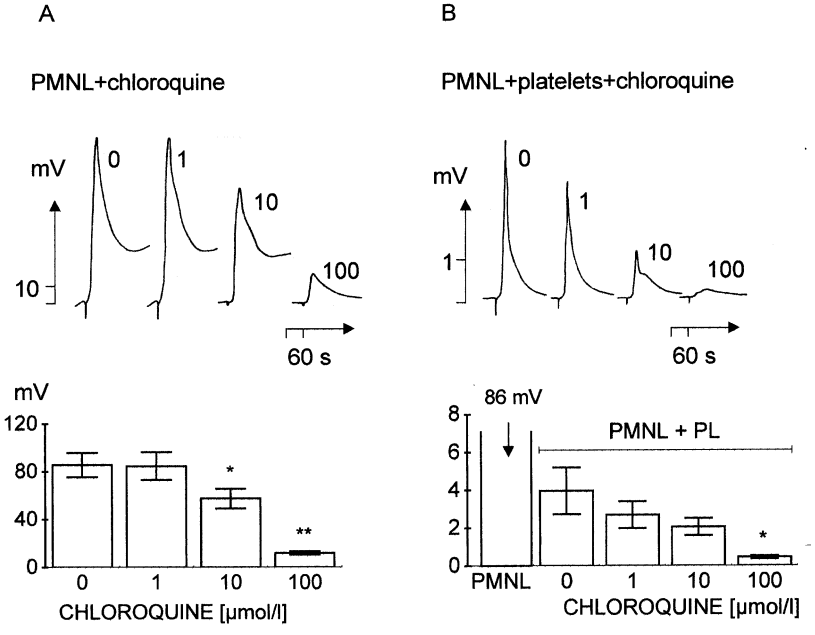

Fig. 2. Isoluminol-enhanced chemiluminescence of A23187-stimulated PMNL measured in the presence of HRP; dose-dependent effect of chloroquine (part A) and of chloroquine + blood platelets (part B). Representative curves, mean ± S.E.M., $n = 8$, $^{*}P < 0.05$, $^{**}P < 0.01$.

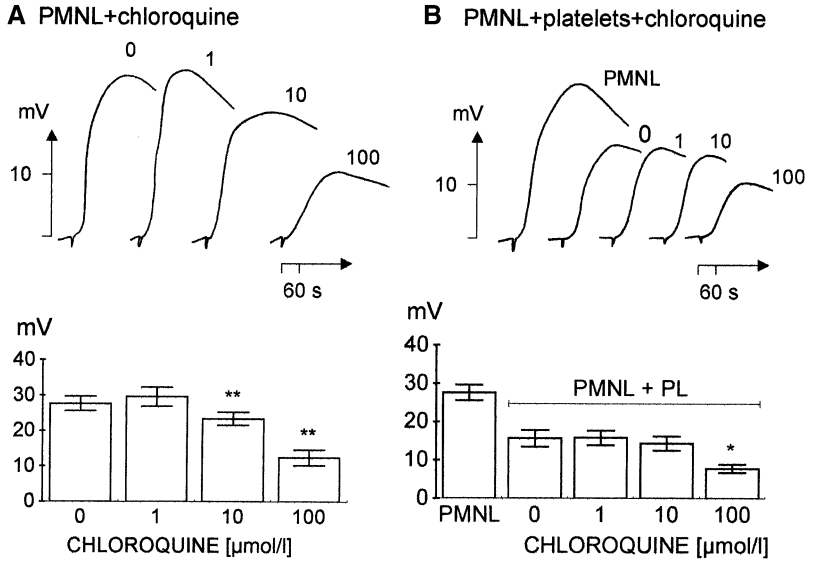

Fig. 3. Luminol-enhanced chemiluminescence of A23187-stimulated PMNL measured in the presence of SOD and CAT; dose-dependent effect of chloroquine (part A) and chloroquine in combination with blood platelets (part B). Representative curves, mean ± S.E.M., $n = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$.

After addition of 10 and 100 μmol/L chloroquine, the intracellular chemiluminescence, enhanced with luminol and measured in the presence of SOD and CAT, was decreased from 27.61 ± 2.05 (control) to 23.39 ± 1.86 and 12.41 ± 2.21 mV, respectively (Fig. 3, Panel A). Platelets lowered control chemiluminescence to 15.66 ± 2.18 mV (Panel B). In this assay system platelets were less effective than 100 μmol/L chloroquine. The lowest chemiluminescence (7.81 ± 1.07 mV) was measured in the presence of both platelets and chloroquine in the concentration of 100 μmol/L.

To separate the effect of chloroquine from that of platelets, inhibition of PMNL chemiluminescence was expressed as percentage of control. This calculation showed that the intensity of chloroquine-mediated inhibition of chemiluminescence was not modified by platelets (Table 1). It is evident that there was no significant difference between reduction of the chemiluminescence signal found in the absence (Column PMNL) and in the presence (Column PMNL + platelets) of platelets. Moreover, the extracellular and intracellular effects of chloroquine were found to be comparable because isoluminol and luminol chemiluminescence were inhibited in parallel.

On the other hand, platelet-induced inhibition was more evident in the extracellular space, and the reduction of isoluminol chemiluminescence reached ca 96% independently of chloroquine concentration (Table 2). Decrease in the intracellular chemiluminescence, observed in the presence of platelets, varied between 30% and 45% of the total and declined slightly with increasing chloroquine concentration.

The results presented in Figs. 4 and 5 indicated involvement of platelet serotonin in inhibition of PMNL chemiluminescence. Activation of platelets with Ca^{2+}-ionophore

Table 1
Chloroquine induced inhibition of PMNL chemiluminescence in the absence and in the presence of blood platelets

	Chloroquine [μmol/l]	Percentage of control	
		PMNL	PMNL+platelets
Isoluminol + HRP	0	100	100
	1	101.89 ± 11.55	89.62 ± 13.98
	10	70.88 ± 7.64	68.93 ± 8.57
	100	15.30 ± 2.22	21.65 ± 6.53
Luminol + SOD + CAT	0	100	100
	1	107.55 ± 6.59	103.57 ± 5.80
	10	84.79 ± 2.55	92.80 ± 1.78
	100	45.42 ± 7.01	52.04 ± 7.10

Control (= 100%) represents the chemiluminescence in the absence of chloroquine. Mean ± SEM, n = 5-8. HRP − horseradish peroxidase, SOD − superoxide dismutase, CAT − catalase.

Table 2
Platelet induced inhibition of PMNL chemiluminescence in the presence of different concentrations of chloroquine

	Chloroquine [μmol/l]	Percentage of control	
		PMNL	PMNL+platelets
Isoluminol + HRP	0	100	4.67 ± 1.11
	1	100	4.04 ± 1.03
	10	100	3.49 ± 0.55
	100	100	4.19 ± 0.97
Luminol + SOD + CAT	0	100	55.77 ± 5.00
	1	100	54.58 ± 7.20
	10	100	61.07 ± 5.31
	100	100	70.29 ± 14.25

Control (= 100%) represents the chemiluminescence of PMNL at the given chloroquine concentration. Mean ± SEM, n = 5-8. HRP − horseradish peroxidase, SOD − superoxide dismutase, CAT − catalase.

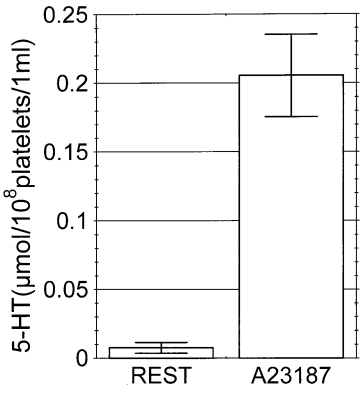

Fig. 4. Concentration of serotonin (5-HT) released from blood platelets after their stimulation with Ca^{2+}-ionophore A23187, calculated to platelet number and sample volume used in the chemiluminescence assay. Mean ± S.E.M., $n = 10–12$; REST = spontaneous release.

A23187 increased extracellular serotonin concentration to 0.206 ± 0.030 μmol/10^8 platelets/1 mL (Fig. 4), with donor variance between 0.123 and 0.355 μmol/L. This amount of liberated serotonin could possess sufficient inhibitory effect because addition of serotonin to PMNL in concentrations of 0.1, 0.5, and 1 μmol/L reduced their chemiluminescence from 140.76 ± 13.72 mV (control) to 114.59 ± 15.52, 43.36 ± 11.48, and 20.57 ± 4.06 mV, respectively (Fig. 5).

Chloroquine dose-dependently liberated serotonin from platelets (Table 3). Starting with 1 μmol/L concentration, chloroquine potentiated the releasing effect of A23187, and at higher concentrations used (10 and 100 μmol/L) it induced discharge of serotonin from platelets. In resting platelets, chloroquine (100 μmol/L) increased the extracellular serotonin concentration from 0.007 (control) to 0.107 μmol/10^8 platelets/mL. In A23187-stimulated and chloroquine-treated platelets, the maximum amount of serotonin liberated was 0.262 μmol/10^8 platelets/mL.

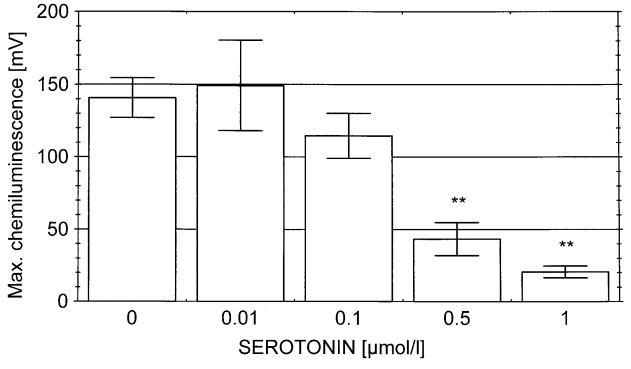

Fig. 5. Dose-dependent inhibition of PMNL chemiluminescence by increasing concentrations of serotonin stock solution. Chemiluminescence was induced with A23187, enhanced with isoluminol, and measured in the presence of HRP. Mean ± S.E.M., $n = 6$, $^{**}P < 0.01$.

Table 3
Dose-dependent effect of chloroquine on serotonin liberation from unstimulated and Ca^{2+}-ionophore A23187 stimulated blood platelets

Chloroquine [μmol/l]	Platelet serotonin [% of liberated]	
	No stimulus	A23187
0	1.18 ± 0.67	59.96 ± 6.55
1	0.97 ± 0.46	71.92 ± 5.03**
10	8.87 ± 2.51*	74.42 ± 5.74**
100	33.93 ± 7.08**	82.60 ± 4.53**

Mean ± SEM, n = 5-8, *p < 0.05, **p < 0.01 (vs chloroquine 0).

4. Discussion

4.1. Detection of reactive oxygen species by the chemiluminescence method

The presented results revealed a significantly reduced concentration of PMNL-derived oxygen metabolites in the presence of chloroquine and blood platelets. Their detection by means of chemiluminescence is considered to be highly sensitive [21–24] and complex because this method can visualize both radicals produced by plasma membrane into extracellular space as well as radicals originated intracellularly on membranes of specific granules [18,25,26]. Because isoluminol, in contrast to luminol, is not able to pass biological membranes [18,24], two assay systems were applied in our experiments. They enabled us to record separately the extra- and intracellular part of the chemiluminescence signal and thus to locate the site of action for chloroquine and blood platelets. As confirmed by the effect of extracellular scavengers (Fig. 1), chemiluminescence enhanced with isoluminol (System I) detected reactive oxygen species localized outside PMNL, whereas chemiluminescence enhanced with luminol and measured in the presence of SOD and CAT (System II) was predominantly connected with intracellularly produced oxidants.

Detection of chemiluminescence by a lumiaggregometer equipped with highly sensitive luminescence detector is considered to be an eligible method [19,27]. The registered signal possessed all main characteristics of chemiluminescence: it arose with neutrophil activation, was luminol- and peroxidase-dependent, and was blocked in the presence of radical scavengers [7,28]. The chemiluminescence curves, received in the presence of ionophore, were comparable to those detected by luminometer, as demonstrated by their culmination within 1–4 min of stimulation and by close peak times of extra- and intracellular chemiluminescence [18,29].

4.2. Inhibition of chemiluminescence by blood platelets

Platelets, co-incubated with PMNL in the physiological ratio 50:1, effectively decreased chemiluminescence stimulated with A23187, and their effect was comparable with inhibition produced by 100 μmol/L chloroquine. As we

found earlier, platelets, in the presence of Ca^{2+}-ionophore A23187, become activated, and the stimulation is not considerably counteracted by chloroquine [16]. Therefore, the observed inhibition of PMNL chemiluminescence could be explained by the effect of substances released from platelets during their activation [7,28]. Particularly, involvement of serotonin seems to be highly acceptable because it possesses antioxidative and scavenging effects [4,30–32], and its concentrations, achieved in supernatants of A23187-activated platelets, were found to be sufficient for inhibition of chemiluminescence (Figs. 4 and 5). Values of serotonin released from donor platelets were in the range 0.125–0.355 μmol/L, and the inhibition of chemiluminescence started at 0.1 μmol/L concentration (decrease by 18.6%) and rose to 69% inhibition with 0.5 μmol/L serotonin. Moreover, the reducing effect of 0.1 μmol/L serotonin was also found in chemiluminescence stimulated with PMA (by 36%) or with opsonised zymosan (by 11%) [4,33]. However, the effectiveness of these concentrations of serotonin was lower than the inhibition observed in the presence of activated platelets. Therefore, the participation of other platelet-derived substances is suggested. Under the effect of chloroquine, the extracellular concentration of serotonin was only slightly increased from 0.206 to 0.262 μmol/L, so that the protective effect of platelets on stimulated chemiluminescence was not potentiated (Table 2).

Further platelet-derived substances, potentially involved in inhibition of chemiluminescence, are (a) β-thromboglobulin, adenosine, adenosine monophosphate, P-selectin, and platelet-derived growth factor, which inhibit superoxide anion formation [3,5,6]; (b) an unidentified high molecular weight substance decreasing liberation of myeloperoxidase [34]; and (c) radical scavengers, e.g. histamine, CAT, and glutathione peroxidase [35]. Finally, platelets contain high amounts of taurine [36], which is able to eliminate HOCl. However, its decreasing effect on chemiluminescence occurred at 20 mmol/L concentration [19]. Therefore, involvement of taurine in platelet-induced inhibition has yet to be analyzed in more detail.

Blood platelets were found to inhibit both extra- and intracellular chemiluminescence (as recorded by assay Systems I and II). This may indicate (a) their ability to reduce the risk of tissue damage in the proximity of activated PMNL and (b) interference of platelets with signal transduction processes in PMNL. Although reduction reactive oxygen species in the extracellular space could be explained by the effect of the above-mentioned compounds liberated from platelets, the mechanisms involved in platelet-induced decrease of intracellular chemiluminescence remain unknown.

4.3. Chloroquine-induced inhibition of chemiluminescence

Two chemiluminescence assay systems used in our experiments enabled us to discriminate the extra- and intracellular effect of chloroquine. Chloroquine was found to effectively decrease the concentration of reactive oxygen species in the extracellular space, as well as the amount of radicals originated inside PMNL. These effects do not seem to arise from neutralization of reactive oxygen species by the scavenging activity of this drug because chloroquine had no effect on superoxide anion and hydrogen peroxide produced in cell-free systems [9,37–39] and did not reduce the concentration of peroxyl radical (unpublished observations) formed by thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride [for method see 21,22]. More likely, inhibition of chemiluminescence may result from chloroquine-induced reduction of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and from subsequently decreased superoxide anion production [8,9,37,38,40–43].

Chloroquine partitioned in blood fractions and its intracellular concentrations [44,45] showed a significant accumulation of this drug in phagocytes. Cellular concentration of chloroquine (0.1–0.3 mmol/L) was approximately 100 times higher than therapeutic plasma levels. Subsequent alkalinization of the intralysosomal compartment (resulting from the weak base character of chloroquine) could impair granule/plasma membrane fusion and in this way hinder the active NADPH oxidase complex to assembly [38]. Inhibition of phospholipid methylation [46], of phospholipases, and of protein phosphorylation [9] as well as dissociation of inositol 1,4,5-trisphosphate from its receptor and reduced calcium release from intracellular stores [47] are discussed as further mechanisms potentially involved in chloroquine-induced decrease of NADPH oxidase activity.

Because chloroquine significantly decreased extra- and intracellular chemiluminescence at higher concentrations than those detected during antimalarial treatment [48], reduced production of reactive oxygen species could not impair antimicrobial activity of PMNL. However, in patients with rheumatoid arthritis, the concentration of this drug in PMNL is comparable to the concentration achieved *in vitro* during 60 min incubation of PMNL with 100 μmol/L chloroquine [45]. This makes the involvement of decreased oxidant formation in the anti-inflammatory effect of chloroquine rather conceivable.

4.4. Joint action of chloroquine and platelets

It is evident from the results presented here that chloroquine and platelets cooperated in the inhibition of chemiluminescence and from their common effect was much more extensive than reduction induced by these inhibitors separately. The addition of two effects rather than their mutual potentiation was assumed because neither the chloroquine effect was potentiated in the presence of platelets nor inhibition by platelets was intensified by chloroquine (Tables 1 and 2). Chloroquine and platelets could interfere with radical formation at different sites because effectiveness of this drug was not altered in the presence of platelets. A coincidence of two different mechanisms can also be suggested:

first, the direct scavenging effect of platelets [4,30–32] and, second, the inhibition of NADPH oxidase by chloroquine [8,9,37,38,40–43]. Nevertheless, the existence of a common site of action for both platelets and chloroquine might be taken into account, owing to the fact that the intracellular effect of platelets was slightly declined with increasing concentration of chloroquine (Table 2).

The effective cooperation between chloroquine and blood platelets indicated that under *in vivo* conditions the effect of a drug on one type of cells could be modified in the presence of cells of another type. Therefore, for accurate prediction of drug effect in the whole organism the use of multicellular test systems seems to be pertinent.

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