Hypochlorous acid/N-chloramines are naturally produced DNA repair inhibitors

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Human mononuclear leukocytes (HML) respond to oxidative DNA damage by activation of ADP ribosylation and initiation of DNA repair synthesis (i.e. unscheduled DNA synthesis, UDS), whereas neutrophils do not. When neutrophils are added to HML cultures in ratios up to 4:1 ADP ribosylation becomes inhibited to ~50–60%. The ability of neutrophils to inhibit HML ADP ribosylation was shown to be dependent on H2O2, chloride ions and myeloperoxidase, which in turn are factors known to govern HOCI and N-chloramine production by phagocytic cells. HOCI and a model N-chloramine, chloramine T, were shown to give a dose-dependent inhibition of DNA repair using four independent estimates, namely ADP ribosylation, UDS and the repair of DNA strand breaks estimated by nucleoid sedimentation and alkaline elution profiles. All the DNA repair measurements used on HML were inhibited ~70–80% by 100 μM doses of HOCI or chloramine T, which was considered a biologically relevant dose because: (i) viable neutrophils equal in concentration to those found in blood could easily produce 100 μM levels in short-term culture; (ii) 100 μM doses of these agents were not acutely cytotoxic judged by trypan blue stained cells after 30–60 min exposure and under the conditions used for assay, but yet they abolished 86–95% of the growth response of HML to phytohemagglutinin.

Introduction

DNA repair has been repeatedly implicated as a cellular event important to the carcinogenic process (for reviews see 1,2). The evidence has come mainly from autosomal recessive chromosome instability syndromes such as xeroderma pigmentosum, Bloom’s syndrome and ataxia telangiectasia, which are both DNA repair-deficient and cancer prone disorders (3,4). However, this laboratory and others (5–13) have provided data that dominantly inherited cancers or a genetic predisposition for their occurrence also have reduced DNA repair capacity, which has emphasized that not all DNA repair deficiencies can be explained by our current knowledge of inherited defective DNA repair genes. In an effort to explain this apparent anomaly we have sought to identify a mechanism that might regulate DNA repair by a post-transcriptional alteration of the DNA repair genes.

It is generally well recognized that phagocytes are a rich source of endogenously produced reactive oxygen species such as H2O2, O2•-, *OH, HOCI and N-chloramines (14). Reactive oxygen species are thought to be involved in both the initiation and promotion phases of carcinogenesis (15,16). This laboratory (12,17) and others (reviewed in 15,16) have shown that H2O2, O2•- and *OH can induce cellular DNA damage and activate the DNA repair process, but comparable data for HOCI and N-chloramines, although very limited (14,18,19), have not confirmed that they act like H2O2, O2•- or *OH in cellular systems.

One feature of HOCI and N-chloramines that has been established in the literature is their ability to react with sulfhydryls, a mechanism identified for this type of prooxidant as explaining their high degree of cytotoxicity (14,19). Poly-(ADP-ribose) transferase (ADPRT) is a nuclear enzyme that becomes activated by DNA strand breaks, ADP-ribosylates chromatin proteins and, as such, participates in the DNA repair process (20,21). The ADPRT enzyme has been cloned, sequenced and three regions identified: i.e. a DNA binding domain, a catalytic domain and a NAD binding domain (22).

There are two zinc fingers in the DNA binding domain and each one contains three cysteine residues where the zinc binds (23), which enables this enzyme to bind to DNA and to participate in DNA repair. Hence, DNA repair that involves ADPRT is sensitive to cellular reduction/oxidation balance. This laboratory has confirmed the sensitivity of DNA repair to the presence of sulfhydryls by up- and down-regulating this process by exposure to reduced and oxidized glutathione (24).

Here we have examined in depth the hypothesis that DNA excision repair can be inhibited by phagocyte-produced HOCI and N-chloramines at physiologically relevant doses.

Materials and methods

Materials

N-Acetoxy-2-acetylaminofluorene (NA-AAF) was supplied by Chemsyn Science Laboratories. Radiolabeled [2,8-3H]-adene NAD and [3H]NAD (24 and 5 Ci/mmol) came from DuPont and Amersham respectively. Fisher Scientific supplied sodium hypochlorite. Sigma was the commercial source for 3-amino-1,2,4-triazole, A'-chloro-p-toluene sulfonamide (sodium salt, chloramine T), phorbol-12-myristate-13-acetate (PMA), H2O2, hydroxyurea and sodium azide. Neutrophil isolation medium and mononuclear leukocyte isolation medium were from Cardinal Associates Inc. (Sante Fe, NM) and Organon Teknika Corporation (Durham, NC) respectively.

Blood cell preparation

Peripheral blood samples from apparently healthy volunteers were obtained by venous puncture and collected into heparinized vacutainers (143 USP U/10 ml tube). When only human mononuclear leukocytes (HML) were desired the blood was layered on top of a commercially available density cushion (1.077 gm/ml; Organon Teknika) and the cells were isolated and washed using RPMI 1640 medium in the conventional manner (25). When both HML and neutrophils were needed the cell fractions were simultaneously

*Abbreviations: ADPRT, poly(ADP-ribose) transferase; NA-AAF, N-acetoxy-2-acetylaminofluorene; PMA, phorbol-12-myristate-13-acetate; HML, human mononuclear leukocytes; KRPG, Krebs-Ringer phosphate buffer with glucose, pH 7.4; UDS, unscheduled DNA synthesis; PHA, phytohemagglutinin.
isolated by layering the blood sample on top of neutrophil isolation medium (Cardinal Associates) and carrying out all steps in the density gradient isolation using Krebs-Ringer phosphate buffer with glucose, pH 7.4 (KRPG; 26), according to the procedure of Nathan (27).

**Estimation of viability by trypan blue exclusion**

Regardless of the isolation method used for blood cell fractionation, HML were always resuspended in 10–20% serum- or plasma-supplemented RPMI 1640 medium, pelleted, and then resuspended again in either physiological saline or KRPG buffer for treatment with either HOCl or chloramine T. HOCl concentration was determined from the E235 = 100/M/cm. HML viability was monitored by cellular exclusion of trypan blue (0.2% isotonic solution + 5% serum) after 15 min incubation with the dye at 37°C. The stained (non-viable) and unstained (viable) HML were recorded 30–60 min after each treatment as per cent viability (i.e. unstained HML).

**ADPRT assay**

The procedure was adapted from the permeabilized cell technique of Berger (28) with modifications as previously described (11). Duplicate samples of 1X10^6 HML in the presence of 0–4X10^6 neutrophils were cultured in 1 ml KRPG buffer for 30 min at 37°C in the presence of PMA (25 ng/ml). After this co-incubation the HML + neutrophil mixtures were harvested by centrifugation, permeabilized and ADPRT activity determined by a radiometric procedure as described in detail elsewhere (11). In other experiments duplicate HML samples of 1X10^6 per ml KRPG buffer were directly treated with 0–100 μM dose ranges of HOCl or chloramine T from 0 to 100 μM for 30 min at 37°C, which was then followed immediately by treatment with standardized doses of either H2O2 (100 μM) or PMA (25 ng/ml) for another 30 min before analysis of ADPRT activity as already outlined above (11).

**Unscheduled DNA synthesis (UDS) assay**

Approximately 5X10^6 HML were exposed to a standardized 10 μM dose of NA-AAF for 1 h at 37°C in 5 ml 20% autologous plasma-supplemented RPMI 1640 medium. The NA-AAF-treated HML were harvested, resuspended in physiological saline and next immediately exposed to a dose range of HOCl or chloramine T from 0 to 100 μM for 30 min at 37°C. The NA-AAF + HOCl- or + chloramine T-treated HML were again harvested by centrifugation and then incubated overnight at 37°C in fresh 20% autologous plasma-supplemented RPMI 1640 medium containing 10 μM hydroxyurea and 10 μM auranofin (Sigma). Finally, the HML were harvested and DNA extracted and immobilized onto nitrocellulose filters, the radioactivity determined by scintillation counting, the DNA quantified by the diphenylamine procedure and UDS calculated as already described (15,16,20).

**Nucleoid sedimentation assay**

The repair of H2O2-induced DNA damage in the presence of chloramine T was evaluated using nucleoid sedimentation. HML (5X10^6/ml) were cultured in physiological saline in the presence or absence of 10 μM H2O2 for 30 min at 0°C. Next ± 10 μM chloramine T was added at 0°C, the mixture continued for another 120 min at 37°C and 50 μl aliquots (250,000 HML) taken at various time points during this period for the measurement of DNA damage and repair. Nucleoid bodies were prepared and centrifuged at 25,000 r.p.m. in a Beckman SW 50.1 rotor for 30 min at 4°C after layering on top of 15–30% sucrose gradients as described (24). The sedimentation of nucleoids was recorded as a percentage of the untreated control nucleoid sedimentation distance.

**Alkaline elution assay for single-strand DNA breaks**

HMLs were prepared and exposed to 100 μM H2O2 + 100 μM chloramine T as described in the method for nucleoid sedimentation. The alkaline elution assay was carried out as described by Kohan and co-workers (29), with modifications to measure the unlabelled DNA by microfluorometry (30). Briefly, 2X10^6 cells in ice-cold phosphate-buffered saline were layered onto 2 pore size, 25 mm diameter polycarbonate filters (Millipore), lysed with 4 ml 2 M NaCl, 0.04 M EDTA, 0.2% sarkosyl, 0.5 mg/ml proteinase K, pH 10.0, washed with 2.5 ml 0.02 M EDTA, pH 10.0, and eluted in the dark with 0.01 M sodium EDTA, pH 12.3, at a flow rate of 0.038 ml/min. Fractions were collected every 90 min for 9 h and they represented the single-strand damaged DNA that eluted under the above conditions. After this elution filters were cut into pieces, dissolved in 8 ml eluting solution, vortexed, spun down and the DNA measured in the supernatant, which represented the undamaged fraction of DNA retained on filters. The results were calculated as a per cent of total DNA by the formula: DNA retained on filter (%) = [μg DNA on filter/μg DNA on filter + S μg DNA in fractions eluted]X100.

**HOCl measurement**

Mixed cultures of HML + neutrophils were assayed for the production of HOCl in the extracellular conditioned medium by removal of the cells by centrifugation following the incubation period and immediate trapping of the produced HOCl with taurine (20 mM). Taurine chloramine was then quantified spectrophotometrically by using the conversion of 1 to 1 (ε = 2.28X10^3/M/cm). Details of this procedure have been described by Weiss et al. (31).

**Results**

**Neutrophil modulation of ADP ribosylation in HML**

When H2O2, O2- or *OH generating systems are used to damage DNA in HML there is activation of ADP ribosylation (12,17). Contrarily, terminally differentiated neutrophils do not respond to the induction of DNA damage by activation of ADP ribosylation (32). For example, in our laboratory when 4X10^6 human purified neutrophils were exposed to a standardized dose of 100 μM H2O2 in KRPG buffer for 30 min there was no measurable activation of ADP ribosylation when determined as reported in Materials and methods. These earlier results could be explained by the possibility that neutrophils may produce inhibitors of ADPRT which prevent their activation. In order to test this possibility we have combined, under conditions that permit viable cell culturing, a standardized amount of HML (1X10^6) together with increasing amounts of neutrophils from 0–4X10^6 cells per culture. Next these combined cultures were exposed to PMA to activate ADP ribosylation in HML and to induce the production of oxy radicals by neutrophils. The data shown in Figure 1 show that when HML + neutrophil ratios reached 1:2 (X10^6 cells/ml), which is comparable with the proportion and concentration in blood, HML ADP ribosylation began to become severely inhibited. The respiratory burst induced by PMA exposure of neutrophils was monitored by HOCl production using the taurine trapping technique (31). It was concluded that either the presence of 2X10^6 neutrophils or the production of ~80 μM HOCl or...
N-chloramine was sufficient to cause inhibition of HML ADP ribosylation.

Dependence of neutrophil modulation of ADP ribosylation in HML on myeloperoxidase activity

Myeloperoxidase activity is specific to phagocytes and this enzyme is the only endogenous source of HOCl production (14,33). In an effort to distinguish between whether the HML ADPRT inhibition induced by neutrophils was due to HOCl production or due to the presence of some other inhibiting factor(s) coming from neutrophils we have determined if HML ADPRT was dependent on myeloperoxidase activity. There are three factors known to govern HOCI production by neutrophils, namely H$_2$O$_2$, chloride ions and myeloperoxidase. The data presented in Table I show that: (i) addition to HML + neutrophil cultures of H$_2$O$_2$, which is the substrate for myeloperoxidase and thus stimulates HOCI production, results in a dose-dependent inhibition of HML ADPRT activity; (ii) addition to HML + neutrophil cultures of sodium fluoride, which can compete for chloride and thus inhibit the formation of HOCI, results in a dose-dependent activation of HML ADPRT activity, due to the increased presence of H$_2$O$_2$ due to inhibition of myeloperoxidase, which in turn stimulates ADPRT activity (11); (iii) addition to HML + neutrophil cultures of 3-aminotriazole or sodium azide, which are well-established inhibitors of myeloperoxidase, also activates HML ADPRT activity and thus blocks the ability of neutrophils to inhibit ADPRT. Taken together these data strongly suggest that HOCI, and not some other neutrophil factor, is responsible for inhibition of HML ADPRT activity.

Effects of HOCl and chloramine T on HML viability estimated by trypan blue exclusion

The cytotoxicity of HOCl and N-chloramines is well established (14,19). Hence, it is important to determine that any biochemical effects on DNA repair and cell function induced by these prooxidants are not related to acute cytotoxicity (i.e. trypan blue staining within 30–60 min after exposure). We have observed that HML are very sensitive to HOCl and the model N-chloramine, chloramine T, after 1–2 washes in physiological saline or KRPG buffer. For example, under these conditions the percent HML unstained cells for HOCl were: 25 μM, 96 ± 10%; 50 μM, 86 ± 7%; 75 μM, 67 ± 5%; 100 μM, 55 ± 15%. The corresponding viability values for chloramine T were: 25 μM, 98 ± 10%; 50 μM, 69 ± 8%; 75 μM, 29 ± 5%; 100 μM, 16 ± 12%. However, if HML washed in either saline or KRPG buffer are first suspended in 10–20% serum/plasma-supplemented RPMI 1640 medium, pelleted and then suspended again in saline or KRPG buffer for exposure to HOCl or chloramine T then there is no significant acute cytotoxicity (i.e. estimated as trypan blue stained cells immediately after the exposure period of 30 min) within the dose range 0–100 μM. Consequently, we have monitored and used non-acute cytotoxicity conditions throughout this study.

Effects of HOCl and chloramine T on HML ADP ribosylation

HML ADP ribosylation was activated by treatment with standardized doses of either 100 μM H$_2$O$_2$ or 25 ng/ml PMA and then the ability of chloramine T or HOCl to inhibit ADPRT

**Table I. Dependence of ADPRT activity in HML on H$_2$O$_2$, chloride ions and myeloperoxidase activity**

<table>
<thead>
<tr>
<th>HML + neutrophils (X 10$^6$)</th>
<th>H$_2$O$_2$ (μM)</th>
<th>NaF (mM)</th>
<th>Myeloperoxidase inhibitor</th>
<th>ADPRT activity (% control ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+2$^a$</td>
<td>Control</td>
<td>100±14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^a$</td>
<td>10 μM</td>
<td>77±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^a$</td>
<td>20 μM</td>
<td>41±5</td>
<td></td>
<td></td>
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<tr>
<td>1+2$^a$</td>
<td>30 μM</td>
<td>36±6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^a$</td>
<td>40</td>
<td>26±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^b$</td>
<td>100</td>
<td>30±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+1.9$^b$</td>
<td>100 Control</td>
<td>100±17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+1.9$^b$</td>
<td>100 10</td>
<td>114±12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+1.9$^b$</td>
<td>100 50</td>
<td>163±20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+1.9$^b$</td>
<td>100 100</td>
<td>174±23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^c$</td>
<td>100</td>
<td>Control</td>
<td>100±9</td>
<td></td>
</tr>
<tr>
<td>1+2$^c$</td>
<td>100 10 mM 3-AT</td>
<td>139±13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^c$</td>
<td>100 50 mM 3-AT</td>
<td>220±37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^c$</td>
<td>100 10 μM azide</td>
<td>650±57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2$^c$</td>
<td>100 100 μM azide</td>
<td>710±71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The cells were treated with 0–100 μM H$_2$O$_2$ in KRPG buffer for 1 h at 37°C. The per cent of control determinations was calculated by dividing the ADPRT activity present in HML + neutrophil cultures by the ADPRT activity present in HML cultures only. There is no ADPRT activity in neutrophils. The ADPRT activity of HML + neutrophils (1 ± 2 х 10$^6$) treated with 100 μM H$_2$O$_2$ ranged from 41 to 615 c.p.m. (9.1–13.6 pmol) TCA-precipitable radioactive NAD.

$a$The cells were first washed in 0.1 M phosphate buffer, 1.5 mM MgSO$_4$, pH 7.4, treated with 100 μM H$_2$O$_2$ and then incubated in the presence of increasing amounts of NaF for 1 h at 37°C.

$b$The cells were first washed in 0.1 M phosphate buffer, 1.5 mM MgSO$_4$, pH 7.4, treated with 100 μM H$_2$O$_2$ and then incubated in the presence of increasing amounts of NaF for 1 h at 37°C.

$^c$The cells were first washed in 0.1 M phosphate buffer, 1.5 mM MgSO$_4$, pH 7.4, treated with 100 μM H$_2$O$_2$ and then incubated in the presence of increasing amounts of NaF for 1 h at 37°C.

Fig. 2. Effect of chloramine T and HOCl on H$_2$O$_2$-activated ADPRT activity in HML. HML were first treated with 100 μM H$_2$O$_2$ and then immediately exposed to the indicated doses of chloramine T or HOCl. Data points and bars represent mean ± SD.

Fig. 3. Effect of chloramine T and HOCl on PMA-activated ADPRT activity in HML. HML were first treated with 25 ng/ml PMA and then immediately exposed to the indicated doses of chloramine T or HOCl. Data points and bars represent mean ± SD. Reproduced from Biochimie, 77, 390 (1995).
was assessed by addition of these agents to HML cultures for 30 min as described in Materials and methods. Both chloramine T and HOCl gave a dose-dependent inhibition of ADP ribosylation regardless of whether it was induced by H$_2$O$_2$ or PMA (Figures 2 and 3). About 70–80% inhibition of ADPRT activity was observed at doses of 100 μM HOCl or chloramine T. These doses and the effects on HML ADPRT activity were comparable with those produced physiologically by neutrophils (Figure 1).

Effects of HOCl and chloramine T on other estimates of DNA repair

DNA excision repair is a cellular process involving several enzymatic events (1–3,20,21) and it is possible that although ADPRT is implicated in DNA repair (20,21), the inhibitory effects of HOCl or chloramine T on ADPRT might not be reflected in other quantitative estimates of DNA repair. We have confirmed that this is not the case using three other independent estimates of DNA repair, namely UDS and the repair of DNA strand breaks estimated by nucleoid sedimentation and alkaline elution profiles. NA-AAF-induced UDS was dose dependently inhibited by 67 and 75% at doses of 100 μM chloramine T and HOCl respectively (calculated from Figure 4). This inhibition was apparently irreversible, since the cells were washed free of these two prooxidants before estimating UDS over an 18 h incubation period. Similar results were obtained when the repair of H$_2$O$_2$-induced DNA strand breaks measured up to 2 h after H$_2$O$_2$ treatment by either nucleoid sedimentation (Figure 5) or alkaline elution (Figure 6) was inhibited by exposure to 100 μM chloramine T. Furthermore, the effects of HOCl and chloramine T on DNA repair appear to be quite independent of any direct effects on DNA damage induction, because neither agent could be shown to induce significant DNA damage in HML in short-term culture (Figures 5–7).

Effects of HOCl and chloramine T on HML PHA responsiveness

The effect of DNA repair inhibition caused by HOCl or chloramine T exposure on the function of HML was examined by quantifying the growth induced by the polyclonal mitogenic stimulus PHA. The data in Table II clearly demonstrate that there was a dose-dependent inhibition of the growth response to PHA after exposure to 50–100 μM doses of HOCl or chloramine T and that the level of inhibition was nearly complete, being 86–95% compared with the control levels at doses of 100 μM. These data support the hypothesis that DNA repair inhibition by HOCl/A'-chloramine type products results in a proportionate loss of immune cell (i.e. HML) responsiveness without directly inducing DNA damage (Figure 7), but by permitting its accumulation (Figures 5 and 6).
This study presents evidence that neutrophil production of be reduction/oxidation-sensitive biochemical processes. Four independent measures of cell (i.e. HML) responsiveness. These measures, including ADPRT activity (24) and UDS (24), as well as the responsiveness of HML (36,37), have been shown to be reactive with sulfhydryl amino acid-containing proteins and both ADPRT activity (24) and UDS (24), as well as the responsiveness of HML (36,37), have been shown to be reduction/oxidation-sensitive biochemical processes.

Discussion

This study presents evidence that neutrophil production of HOCl or N-chloramines compromises DNA repair and immune cell (i.e. HML) responsiveness. Four independent measures of DNA repair (i.e. ADPRT activity, UDS and the repair of DNA strand breaks estimated by nucleoid sedimentation and alkaline elution profiles) all gave dose–response curves for DNA repair inhibition by HOCl and the model N-chloramine, chloramine T, where ~70–80% inhibition occurred at 100 μM doses (Figures 2–6). Moreover, 100–200 μM doses of HOCl or chloramine T also resulted in ~60% inhibition of DNA repair when it was produced naturally by cultured neutrophils (Figure 1) or ~86–95% inhibition of growth response when it was added directly to HML cultures activated by the mitogen PHA (Table II). The strong correspondence of these data, combined with the potential for irreversibility (Figure 4) and selectivity (Figure 7) of the effects on DNA repair, have emphasized a consistency and specificity in the data that is convincing of an important physiological role for HOCl and N-chloramines as DNA repair inhibitors.

Moreover, there are two equally compelling explanations for the data reported in this study. One is that HOCl/N-chloramines are very broad spectrum biologically reactive oxidants affecting separate mechanisms in at least three biochemical processes we have studied, namely ADPRT activity, UDS and PHA mitogenic stimulation of HML. This interpretation is supported by the fact that the dose–response curves for inhibition by HOCl/N-chloramines were slightly different for the ADPRT and UDS measures (Figures 2–4), as well as that the observed effects on PHA HML responsiveness (Table II) could have been explained by interference in PHA binding to receptors and not related to DNA damage and repair. The other possibility is that these biochemical processes have a common controlling mechanism that affects all these assay procedures. There are data available to support this contention. Although it is true that the repair of single-strand breaks is not ADPRT dependent (24), the repair of bulky NA-AAF DNA lesions that induce large patch UDS is ADPRT dependent and inhibited by H₂O₂ (24). Likewise, the PHA response of HML is inhibited by ADPRT inhibitors, which demonstrates the dependence on ADPRT (34,35).

In addition, our data are further supported by biochemical considerations. HOCl and N-chloramines are prooxidants expected to be reactive with sulfhydryl amino acid-containing proteins and both ADPRT activity (24) and UDS (24), as well as the responsiveness of HML (36,37), have been shown to be reduction/oxidation-sensitive biochemical processes.

Table II. The effect of pre-incubation with chloramine T or HOCl on the PHA response of HML.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>[H]dThd c.p.m. incorporated/200 000 HML</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>24</td>
<td>11.77±655</td>
<td></td>
</tr>
<tr>
<td>50 μM chloramine T</td>
<td>12</td>
<td>7525±294*</td>
<td></td>
</tr>
<tr>
<td>75 μM chloramine T</td>
<td>12</td>
<td>6781±409*</td>
<td></td>
</tr>
<tr>
<td>100 μM chloramine T</td>
<td>12</td>
<td>537±46*</td>
<td></td>
</tr>
<tr>
<td>50 μM HOCl</td>
<td>12</td>
<td>7109±415*</td>
<td></td>
</tr>
<tr>
<td>75 μM HOCl</td>
<td>12</td>
<td>3572±217*</td>
<td></td>
</tr>
<tr>
<td>100 μM HOCl</td>
<td>12</td>
<td>1627±194*</td>
<td></td>
</tr>
</tbody>
</table>

There was no cytotoxicity 30 min after exposure to HOCl or chloramine T. Details of this method and of the assessment of the PHA response after 96 h in microculture are presented in Materials and methods.

*Mean ± SD, t-test comparison to no treatment, P < 0.001.

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References


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