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ORIGINAL PAPER

Mercurius solubilis: actions on macrophages

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Background: **Macrophages play central roles in homeostasis as well as host defence in innate and acquired immunity, auto-immunity and immunopathology. Our research group has demonstrated the effects of highly diluted toxic substances in macrophages.**
Aim: **To investigate if highly diluted *Mercurius solubilis* (*Merc sol*), can activate or modulate macrophage functions.**
Methods: **We evaluated the effects of *Merc sol* in the 6, 12, 30 and 200 centesimal high dilutions (CH) potencies on mice peritoneal macrophages (*in vitro* and *in vivo*). *Merc sol* was added to mice's drinking water for 7 days (*in vivo* treatment) and animals were euthanised and cells were collected. *In vitro* treatment was performed on macrophages and bone-marrow cell cultures.**
Results: **Macrophages showed activated morphology, both when *Merc sol* was added directly to the cell culture and to drinking water. The *in vitro* experiments showed enhanced morphological activation, increased interferon (IFN)γ release in the supernatant at lower dilutions and interleukin (IL)-4 production at higher dilutions. Increase in nitric oxide and decrease in superoxide (O_2) and hydrogen peroxide (H_2O_2) were also observed. *In vivo* treatment caused a decrease in O_2 and increase in H_2O_2 production by macrophages.**
Discussion: **Taken together, the results allow us to conclude that highly diluted *Merc sol* modulates reactive oxygen species (ROS), reactive nitrogen species (RNS) and cytokine secretion, which are central mediators of the immune system, wound healing and body homeostasis.** *Homeopathy* (2011) **100**, 228–236.

Keywords: *Mercurius solubilis*; Macrophages; Immune system; Reactive species; Cytokines

Macrophages are among the first cells to interact with foreign or abnormal host cells and their products, by virtue of their presence at the portals of entry. Depending on the recognition receptors and stages or states of differentiation, they release various products, including cytokines that mobilise and influence other resident cells in tissues[1] and reactive oxygen and nitrogen species (ROS and RNS) that participate in bacterial killing and have been implicated in inflammation and tissue injury and still can function as signalling cellular.[2] Macrophages also present antigens to T and B-lymphocytes. These cells play several roles, spanning development, wound healing and homeostasis, and yet their destructive potential and secretion products are central to the pathology of acute and chronic inflammatory disease.[3] Their ability to recognise a wide range of endogenous and exogenous ligands, and to respond appropriately, is central to macrophages functions in homeostasis as well as host defence in innate and acquired immunity, auto-immunity, inflammation and immunopathology.[4]

Our research group has demonstrated the effects of highly diluted toxic substances in macrophages.[5,6] Those substances act as immunomodulators, increasing the activity of inducible nitric oxide synthase (iNOS) as well as reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase, consequently producing nitric oxide (NO) and ROS, respectively.[7] Alterations in gene-expression profile mainly in the ones involved with macrophage activation

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were also established due to those highly diluted compounds.[8] We have also described the effects of a complex homeopathic medicine (CHM) in bone-marrow cells, activating stromal and mononuclear cells,[9–11] in co-cultured lymph node cells and macrophages, suggesting that non-toxic therapies using CHMs are a promising alternative approach to the combination treatment of melanomas.[12] Further, we felt the necessity to test the efficacy of other highly diluted substances in immune system modulation.

At room temperature, mercury is a silver-coloured metallic element in fluid form. Organic and inorganic mercury salts are used as antiseptic, preservative, vaccine, etc. Exposure of various mammalian species to mercurial compounds can give rise to immunosuppression and/or autoimmunity.[13] *Mercurius solubilis* (*Merc sol*), a medicine prepared from a complex salt of mercury, is used in homeopathy for the treatment of infectious and suppurative processes.[14] As we have already shown actions of highly diluted substances on immune cells, we decided to investigate whether *Merc sol* acts through the immune system. The use of macrophages as an experimental model may be particularly useful, as they provide a large data set for statistical analysis without the disadvantages of clinical trials. Here, we describe the results of laboratory studies aimed to establish the general behaviour of macrophages challenged with *Merc sol*.

Materials and methods

The method is summarised in Figure 1.

Merc sol, chemical formula $Hg_4ON \cdot H_2NO_3 + NH_4NO_3$, was purchased from authorised agencies, which guarantee the high quality (endotoxin free) and physicochemical composition of their products by the Brazilian Health Ministry. Dilution procedures followed the methodology described in the Brazilian homeopathic pharmacopoeia. In brief, medications were prepared by serial dilutions of 1:100 from original mother tincture in distilled water, followed by several dynamisations (shaking and dilution). Manual shaking (by hand) was performed against a soft pad after each dynamisation. All preparations used here contained a final concentration of 0.1% alcohol. *Merc sol* centesimal high dilutions (CH) and equivalent concentrations are described below:

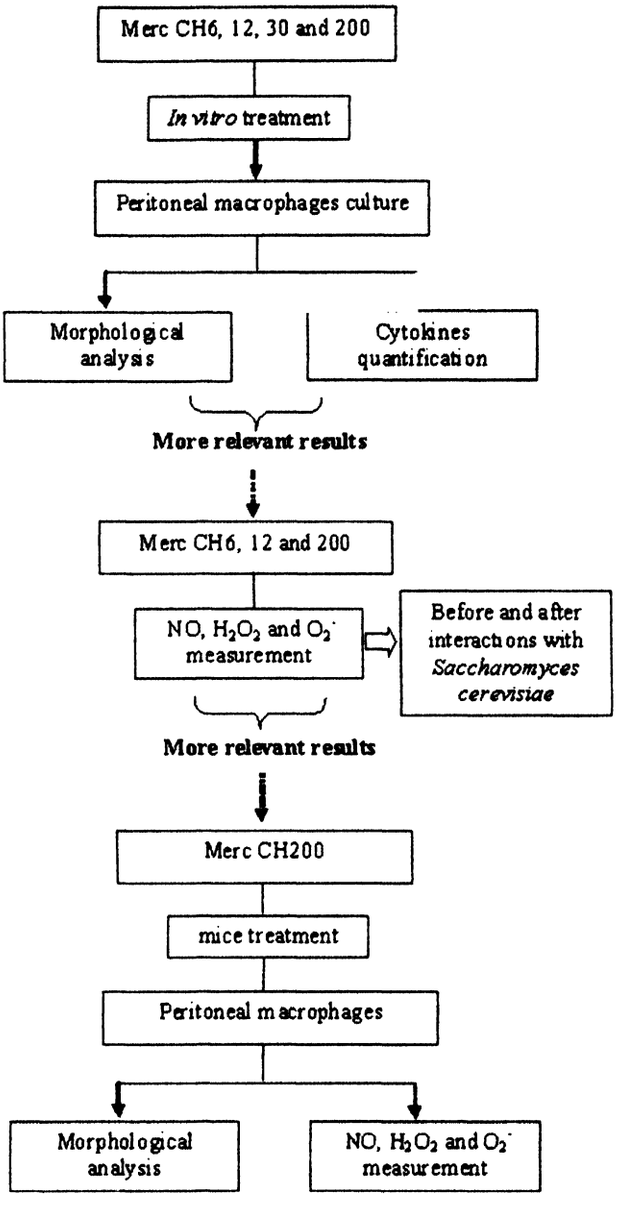

Figure 1 Experiment flowchart.

Concentration (mg/ml)	Merc sol *6 CH*	Merc sol *12 CH*	Merc sol *30 CH*	Merc sol *200 CH*
Merc sol	10^{15}	10^{27}	10^{63}	10^{403}
Hg	8.21×10^{16}	8.21×10^{28}	8.21×10^{64}	8.21×10^{404}

All diluted *Merc sol* used was stored at room temperature and protected from light. An external observer was responsible for coding solutions; the experiment was performed blind. The final dilution of each medication was filtered using a Millipore system (0.22 μm) to yield sterile liquid for addition to cell culture.

Animals

Male albino Swiss mice (6–8 weeks old) weighing 30–35 g were used. The animals were acquired from the Central Animal House of the Federal University of Paraná (UFPR) and received a standard laboratory diet (Purina®) and water *ad libitum*. Mice were subjected to controlled temperature (25 ± 3°C) and a 12-h light/dark cycle (lights on from 6 a.m. to 6 p.m.). We followed the recommendations of the Brazilian National Law n° 6.638.058 November 1979 for the scientific management of animals. The Institutional Animal Care Committee at UFPR approved all related practices. Experiments were carried out in the Inflammatory and Neoplastic Cell

Laboratory, UFPR, which has a toxic waste management programme.

Treatment

In vitro – Macrophages were washed out from mice peritoneal cavities with cold phosphate buffered saline (PBS), pH 7.4. Harvested peritoneal cells were counted using a Neubauer chamber, plated and incubated at 37°C under 5% CO_2 for 15 min. Non-adherent cells were removed by washing with PBS. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 200 U ml^{-1} penicillin, 100 U ml^{-1} streptomycin and 2.6 μg ml^{-1} amphotericin was added to cultures and handled according to the specific procedures required for each experiment. Two hours after plating the cells, the cells were treated with *Merc sol* or control solutions. For morphological and cytokine analyses, cells were treated with *Merc sol* 6, 12, 30 and 200 CH and for reactive species analyses, 6, 12 and 200 CH. Treated groups received 200 μl ml^{-1} (20%) of medication from the same batch of coded substances. After every 24 h, a further dose of 20 μl ml^{-1} was added without replacing the medium. All preparations were vigorously succussed immediately prior to treatment. Experiments were performed at least three times in triplicate.

In vivo – The study was performed blind. Mice were divided into two groups of 10 animals each, treated either with control solution or *Merc sol* 200 CH in drinking water-treated. *Merc sol* one dilution below that administered were kept in 20 ml tubes and a coded by an external observer. Immediately before treatment, the substances were added to a flask containing 180 ml of water. These bottles were succussed and offered to mice in the evening. Each animal received approximately 10 ml day^{-1} for 7 days. All bottles were replaced daily. Mice were subjected to controlled temperature (25 ± 3°C) and a 12-h light/dark cycle (lights on from 06.00 to 18.00) and treatment for 7 days. After this time macrophages were harvested by the method described above from the peritoneal cavity.

Morphological analysis by light microscopy

Macrophages plated and processed for light microscopy. In the case of the *in vivo* treatment group, cells were harvested and plated for 24 h without receiving additional treatment. For *in vitro* experiments, cells were processed after 48 h of culture (see above). Coverslips were washed with PBS, pH 7.4, at 37°C, fixed in Bouin's fixative, stained with Giemsa and dehydrated in acetone and xylol. Slides were mounted with Entelan®.[15] Cells were observed using a Nikon eclipse E200 microscope to differentiate their spreading state. Resting and activated macrophages were differentiated, based on their morphological characteristics.[5] For each coverslip, 100 cells were counted from the total cells plated on each well for each sample. At least six coverslips for each sample were counted. Microscopic fields were randomly chosen. Images were collected using a Nikon Coolpix 4500 digital camera. Data are expressed as percentage of the cells in the spreading state.

Cytokine, ROS and RNS quantification

We evaluated the capacity of macrophages to release tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukins 2, 4, and 5 (IL-2, IL-4 and IL-5). After *in vitro* treatment (5×10^5 cells per well in 96-well tissue culture plates), cytokines in the culture supernatant were estimated. After centrifugation, the peritoneal cells were discarded and the production of cytokines was measured in the supernatant by a Mouse Th1/Th2 cytokine CBA kit (BD—Pharmingen®), according to the manufacturer's instructions. Fluorescence was measured on a FACSCalibur flow cytometer (Becton Dickenson – BD®) equipped with an argon ion laser (488 nm) in FL3 channel. The cytokine concentration was obtained by comparing data with a cytokine curve in the CBA program (BD).

Peritoneal Macrophages (5×10^5 cells per well) were cultured in 96-well plates to determine NO production. After *in vivo* (24 h culture without receiving any additional treatment) and *in vitro* treatment (72 h culture), NO levels in supernatants were determined using Griess reagent.[16] This method estimates the level of nitrite, a stable product derived from a reaction between NO and molecular oxygen, in culture supernatants. In brief, 100 μl of the supernatant were incubated with 100 μl of 1% sulphonamide and 0.1% N-naphthylethylenediamide (in phosphoric acid) at room temperature for 10 min. Lipopolysaccharide (LPS) (50 ng ml^{-1}) and IFNγ (26 U ml^{-1}) were added to cultures for 24 h as positive controls for NO production. The optical density of the samples was subsequently measured at 550 nm on a microplate reader (BIO-RAD®). The nitrite concentration was determined by reference to a standard curve, using sodium nitrite (10–80 μM) diluted in culture medium.

To determine superoxide (O_2^-) production, macrophages (5×10^5 cells per well) were incubated in Hank's Balanced Salt Solution (HBSS) containing ferricytochrome c (80 μM; Sigma®)[17] in the presence or absence of 1 ng ml^{-1} phorbol myristate acetate (PMA). PMA induces O_2^- production by macrophages,[18] and was used as positive control. Absorbance was measured at 550 nm on a microplate reader (BIO-RAD®), and the extinction molar coefficient, $\varepsilon = 2.1 \times 10^4$ M^{-1} cm^{-1}, used to determine reduced ferricytochrome c. Results are expressed as nmol O_2^- per 10^6 cells.

Production of hydrogen peroxide (H_2O_2) by macrophages s following *in vivo* or *in vitro* (48 h) treatment was quantified on the basis of horseradish peroxidase-dependent oxidation of phenol red by H_2O_2.[18] Macrophages (5×10^5 cells per well) were incubated with horseradish peroxidase (15 U ml^{-1}, type IV-A-Sigma) and phenol red solution (194 mg ml^{-1}) dissolved in HBSS at 4°C. PMA (1 ng ml^{-1}) was added as a positive control of H_2O_2 production.[19] Plates were incubated at 37°C for 15 and 30 min, and the reaction terminated by adding 10 ml of 1 M NaOH aqueous solution per well. Absorbance of the cell-free culture supernatant was read at 620 nm on a microplate reader (SLT Lab Instruments 340 ATC®). The H_2O_2 concentration was determined by

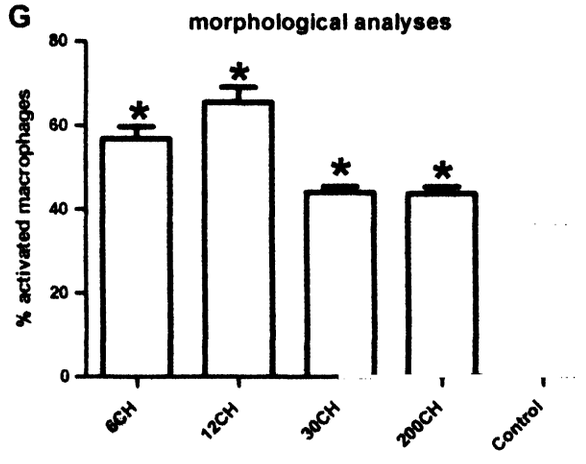

Figure 2 Morphological analysis by light microscopy. Images of peritoneal macrophages observed by light microscopy (original magnification: 1000×). (A): macrophage with resident morphology. (B): macrophage with activated morphology. (C and D): macrophages after 48 h of culture and treated *in vitro* with highly diluted *Merc sol*. (E and F): macrophages of mice treated *in vivo* for 7 days were processed after 24 h of culture without receiving additional treatment. Images of control and treated animals, respectively. (G): percentage of activated macrophages treated with different dilutions of *Merc sol*. Results are expressed as means ± SE of three independent experiments evaluated in a bind way. $^*P < 0.05$ significantly different from control group.

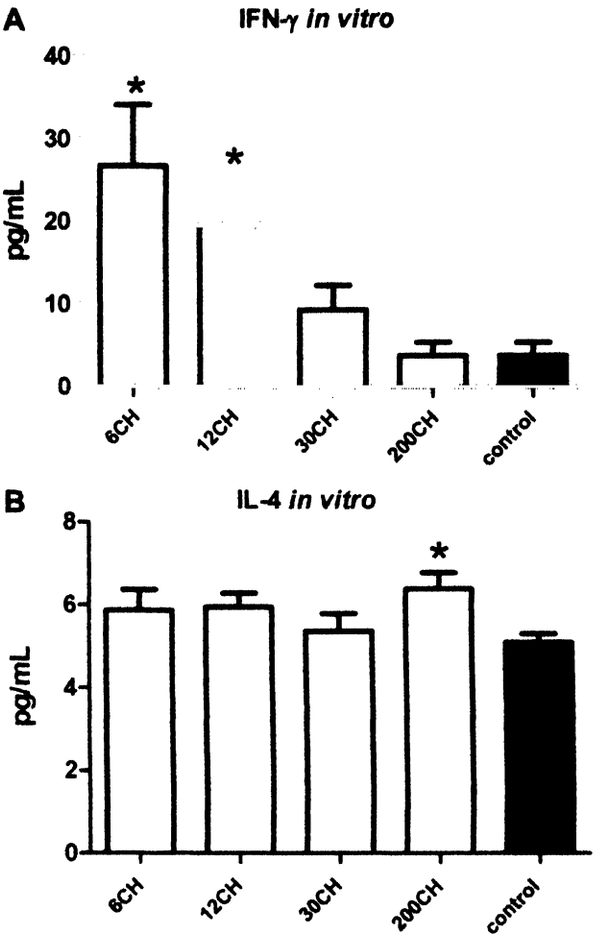

Figure 3 Cytokine production by macrophages treated with diluted *Merc sol*. After *in vitro Merc sol* treatment and 48 h of culture, cytokines released in the culture supernatant were estimated by flow cytometry. (A and B): quantification of IFNγ and IL-4, respectively, released in supernatant. Results are expressed as means ± SE, and $^*P < 0.05$ (increased significantly different from control group).

reference to a standard curve generated using 1–50 nmol H_2O_2 in a solution containing 15 U ml^{-1} peroxidase and 194 mg ml^{-1} phenol red in HBSS.

Production of H_2O_2, O_2^- and NO by macrophages following *in vitro* treatment was additionally quantified after interaction with *Saccharomyces cerevisiae*. In these protocols, macrophages were cultivated for 48 h (for H_2O_2 and O_2^-) or 72 h (for NO) and allowed to interact with yeasts at a ratio of 10:1 for 1 h in a medium without FBS.

Statistical analysis

Experiments were performed at least three times in triplicate. Data from at least three independent experiments were summarised and presented as means ± standard error (SE). Statistical significance was determined using analysis of variance (ANOVA) and Tukey's tests performed by GraphPad Prism 5®. A value of $P < 0.05$ was accepted as statistically significant.

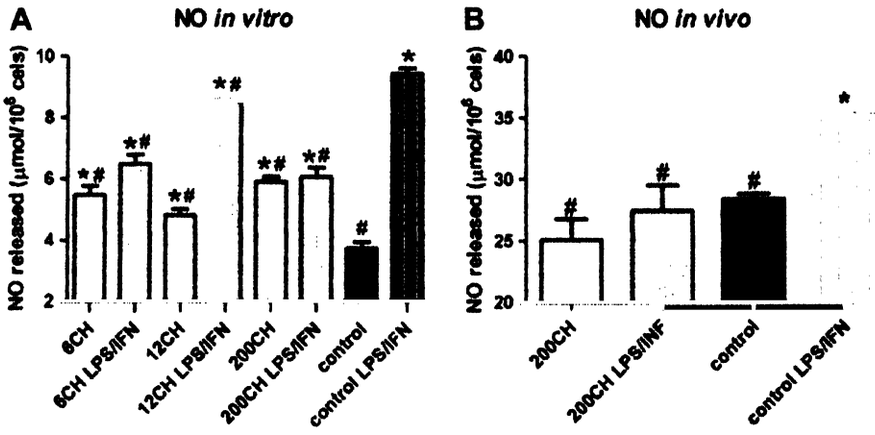

Figure 4 NO production by macrophages. Peritoneal macrophages were culture and NO production was determined. After *in vitro* treatment (72 h of culture) (A) and *in vivo* (24 h of culture without receiving any additional treatment) (B), NO levels in supernatants were determined using Griess reagent. LPS and IFNγ were added to cultures for 24 h as positive controls for NO production. Results are expressed as means ± SE. (*) Increase significantly when compared with control. (#) Decreased significantly when compared with positive control. Significance $P < 0.05$.

Results

Morphological analyses by light microscopy

After mounting the slides, each coverslip received a code by an external observer. For statistical analysis, about 100 macrophages were examined under a light microscope for each coverslip, using an immersion objective. Three independent experiments were performed in triplicate, and consequently, about 5400 cells were subjected to blind analysis. Macrophages' morphological aspects were analysed, leading to their classification as either resident or activated cells (Figure 2(A and B), respectively). The control macrophages morphology varies from rounded cells to characteristically resident macrophages, which exhibited few cytoplasmatic projections and narrow spreading. By contrast, *Merc sol*-treated macrophages showed the typical morphology of the activated stage, such as wider spreading, numerous cellular projections and a large and euchromatic nucleus. Statistical analysis showed that the spreading ability is significantly higher in cells from the *Merc sol*-treated group ($P < 0.05$) confirming that 6, 12, 30 and 200 CH *Merc sol*-induced macrophage activation (Figure 2(G)).

Cytokine quantification

Cytokine quantification experiments after *in vitro* treatment of macrophages showed that *Merc sol*-induced IFNγ production at lower dilutions (6 and 12 CH), while highly diluted (200 CH) *Merc sol* stimulated IL-4 production (Figure 3(A and B), respectively). TNF-α, IL-2 and IL-5 production were not altered.

NO production by macrophages was evaluated in the presence or absence of LPS and IFNγ. After *in vitro* treatment, *Merc sol*-induced NO liberation at 6, 12 and 200 CH (Figure 4(A)). Interestingly, treatment of the positive control groups (treated with LPS and IFNγ) with the above concentrations of *Merc sol* led to decrease in NO production (Figure 4(A)). *In vitro* treatment following macrophage–yeast interactions did not alter NO production. *In vivo* treatment with highly diluted *Merc sol* had no effect on NO production, but when LPS and IFNγ were added the production of NO also decreased in *Merc sol*-treated groups (Figure 4(B)).

The rates of formation of O_2^- in macrophage-culture supernatants, after *in vitro* and *in vivo treatment*, were determined by estimating the reduction of ferricytochrome c. Both *in vitro* (Figure 5(A)) and *in vivo* (Figure 5(B)) treatment decreased O_2^- release by macrophages alone or in the presence of PMA. *In vitro* treatment following macrophages–yeast interactions did not alter O_2^- release.

The production of H_2O_2 by macrophages treated *in vitro* was not altered by *Merc sol* at any of the tested dilutions. After *in vitro* treatment in the presence of yeast, we observed a decrease in H_2O_2 released alone or in the presence of PMA to 6 and 200 CH potencies (Figure 6(A)). With*in vivo* treatment, H_2O_2 release from macrophages was increased (Figure 6(B)). A schematic representation of the effects of *Merc sol* on reactive products released by macrophages is shown (Figure 7).

Discussion and conclusion

We chose macrophages as target cells in our experiments because of their central role in immune modulation, as they participate in mobilization, activation, and regulation of all immune system. Moreover, they interact reciprocally with other cells, while their own properties are modified to perform specialised immunologic functions, particularly *via* secretion of cytokines.[20]

We found that the all tested *Merc sol* dilutions altered macrophages morphology, as evidenced by light microscopy data (Figure 2). We also found that *Merc sol* modulates macrophage functions in the immune system, in terms of cytokine and reactive species production. Lower

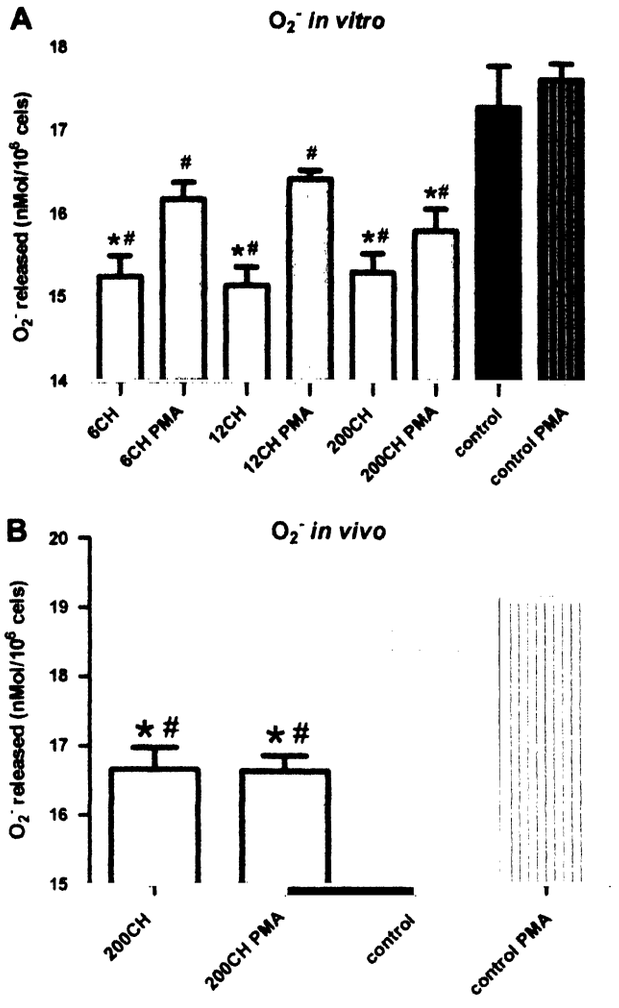

Figure 5 O_2 anion production by macrophages. Macrophages were incubated in HBSS containing ferricytochrome in the presence or absence of PMA. PMA induces O_2 production by macrophages and was thus used as positive control. (A): shows O_2 released after *in vitro* treatment with *Merc sol* and 48 h of culture. (B): shows O_2 released after *in vivo* treatment with Merc. Results are expressed as means ± SE. $^*P < 0.05$ (decreased significantly different from control group). $\#P < 0.05$ (decreased significantly different from positive control with PMA).

Merc sol dilutions increase IFNγ (Figure 3(A)), while the higher ones augmented IL-4 production after *in vitro* treatment (Figure 3(B)). IFNγ promotes Th1 cell responses; it is an important cytokine in host defence against infection by viral and microbial pathogens, together with NO. IL-4 leads to Th2-type humoral immunity and is involved in proliferation and differentiation of activated B-cells.[21] Other studies have shown that low doses of *Merc sol* can cause or exacerbate auto-immune diseases, due to an increase in IFN-γ, IL-4 or T-cells' production.[22,23]

Here, we have shown that high dilutions of *Merc sol* also stimulated the production of cytokines. We have observed that different dilutions of *Merc sol* have different effects, indicating that different potentisations result in different medications. Previous reports using highly diluted substances also have shown differences of cell function according to potency are observed.[24,25] These results may be relevant to homeopathic practice.

After *in vitro Merc sol* treatment, macrophages not only present morphological activation characteristics but also significantly enhanced NO release compared with control (Figure 4(A)). In the activated macrophages NO is produced by iNOS,[2,26] part of the non-specific immune defence mechanism against invading microorganisms, as well as being generated during immune and inflammatory process.

Our results suggest that *in vitro* treatment with *Merc sol* 6, 12 and 200 CH may promote host defence and/or inflammatory responses *via* NO. NO released by macrophages may also activate nuclear transcription factor (NF-κB), which activation seems to be essential for the expression of a large number of cytokines, chemokines and adhesion genes that are critical mediators for inflammatory reactions.[2,26,27] In fact, macrophages treated with *Merc sol* 6 and 12 CH increased IFNγ released, this cytokine also stimulates NF-κB.[26] Interestingly, *Merc sol* treatment of positive control groups also treated with LPS and IFNγ showed reduced NO production (Figure 4(A and B)).

In 2002, Kim, Johnson and Sharma demonstrated that LPS-induced NO production is inhibited in a dose-dependent manner by $HgCl_2$ (5–20 μM) in J774A.1 macrophages. However, further experiments are required to validate this apparent inhibition.

In cells that produce both O_2^- and NO, the near diffusion-limited reaction between NO and O_2^- is likely to occur and results in the formation of peroxynitrite ($ONOO^-$) ($NO + O_2^- \rightarrow ONOO^-$).[2,27] As the NO molecule is small and uncharged, it can traverse the vesicle membrane and would be involved in $ONOO^-$ formation within vesicles in macrophages. $ONOO^-$ oxidises and nitrates a variety of biological targets and is a potential mediator of the cytotoxic effects of NO. Phagocytes also can generate $ONOO^-$ to help to kill pathogens. It can also act as an anti-oxidant defence by preventing an increase in the concentration of O_2^- and H_2O_2.[28]

In vitro treatment with diluted *Merc sol* diminished O_2^- production (Figure 5(A)) and increased NO production. Thus, *Merc sol* potencies may induce the defence functions of macrophages or have an anti-oxidant property. In the case of *Merc sol* 200 CH-treated macrophages, increased IL-4 release might inhibit NADPH oxidase enzyme complex (NOX2), which is an enzyme responsible to O_2 formation, as described by Zhou *et al*.[29]

Phagocytic cells respond to a variety of membrane stimulants *via* the production and release of a number of reactive oxygen reduction products. This response is initiated by the reduction of O_2 to O_2^-.[28] In phagocytic cells, the ROS precursor O_2^- is produced by NOX2 and released outside the cells or mainly released inside phagosomes.[30,31] In fact, all assays with yeast showed a decreased H_2O_2 production when compared with groups without yeast. Significant decrease in H_2O_2 release was also detected in *Merc sol*-treated groups after macrophage–yeast interactions (Figure 6(A)). In phagocytes, NOX2 is localised in both intracellular and plasma membrane, in close association with the $p22^{phox}$ membrane protein. Upon

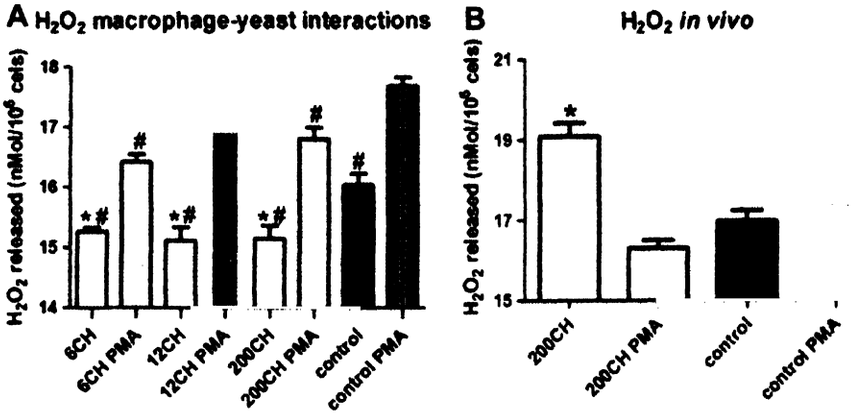

Figure 6 H_2O_2 production by macrophages. Production of H_2O_2 by macrophages was quantified on the basis of horseradish peroxidase-dependent oxidation of phenol red by H_2O_2. Macrophages were incubated with horseradish peroxidase and phenol red solution dissolved in HBSS at 4°C. PMA was added as a positive control of H_2O_2 production. (A): shows H_2O_2 released after *in vitro* macrophage treatment (48 h of culture) followed of macrophage–yeast interactions. $^*P < 0.05$ (significantly different from control group). $\#P < 0.05$ (decreased significantly different from positive control with PMA group). (B): shows H_2O_2 released after *in vivo* treatment. $^*P < 0.05$ (increased significantly different from control group). Results are expressed as means ± SE.

phagocyte stimulation, there is a translocation of NOX2 to the surface as the granule fuses with the phagosomal or the plasma membrane. This fusion is thought to be a key event for the microbicidal activity of NOX2.[30] As a result, H_2O_2 production in treated macrophages probably is directed to the phagosome, consequently decreasing the extracellular liberation in culture supernatant.

After *in vivo* treatment, there was a decrease in O_2^- (Figure 5(B)) and increase in H_2O_2 release (Figure 6(B)). In fact, O_2^- may be dismutated, spontaneously or by O_2^- dismutase (SOD), to H_2O_2 ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$) that can readily diffuse through membranes. This may occur in response to various stimuli, including cytokines and growth factors, involved in the regulation of biological processes as diverse as immune-cell activation[32] and activation of NF-κB.[2] This result suggested that *Merc sol* could be acting in H_2O_2 production and hence, H_2O_2 produced and released after *in vivo*

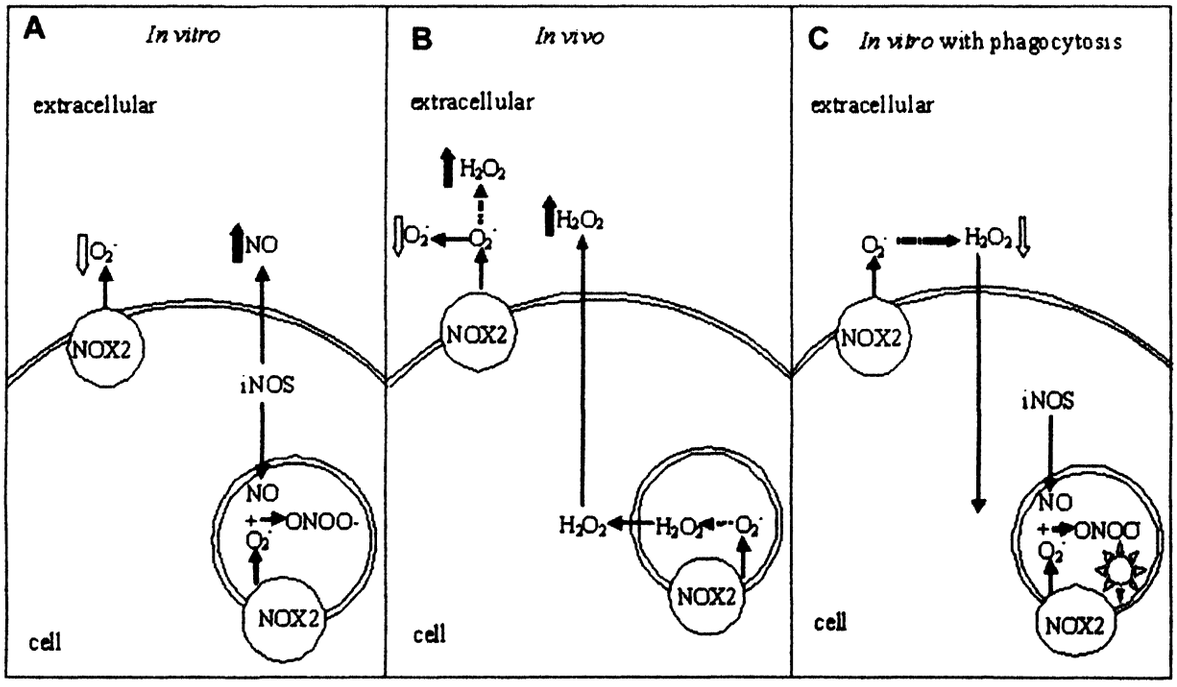

Figure 7 The effects of diluted *Merc sol* on macrophages reactive species release are summarised. (A): *in vitro* treatment increased NO (black arrow) and decreased O_2^- released (white arrow). Higher levels of NO allowed the formation of ONOO within vesicles, decreasing the O_2^- released. (B): *in vivo* treatment also decreased O_2^- (white arrow) but increased H_2O_2 release (black arrow). The O_2^- produced was dismutated to H_2O_2 that could readily diffuse through membranes. (C): after *in vitro* treatment, yeast–macrophage interactions decreased H_2O_2 released (white arrow), because H_2O_2 produced was directed to phagosome, consequently decreasing the extracellular levels in supernatant cultures (white arrow = Increased; black arrow = decreased; sun = yeast).

treatment may act as a signalling molecule regulating other cellular process. In these assays, decreased O_2^- and H_2O_2 release also was observed after addition of PMA and *Merc sol* treatment (Figures 5(A and B) and 6(A), respectively), suggesting a protective effect in the cells from damage caused by oxidative stress.

The chemical and biological interactions of NO and ROS with various biological molecules have important consequences for various immunological and pathological mechanisms conditions.[28] During the inflammatory reactions, pro-inflammatory mechanisms are important to eliminate the causative infectious, toxic or allergenic agents. Diluted *Merc sol* has been shown to modulate macrophages function, as it has increased the production of IL-4, IFN-γ, NO, H_2O_2 and probably $ONOO^-$. However, the inflammatory process once induced, are later down-regulated to allow healing. Therefore, pro-inflammatory and anti-inflammatory mechanisms must be activated spatially and temporally in a finely tuned manner. Highly diluted *Merc sol* can provide a balanced interplay. The results allow us to conclude that in normal situations, *Merc sol*-treated macrophages might balance the Th1 or Th2 immune responses, probably preventing an increase in ROS concentration. After interaction with yeast, macrophages probably concentrate ROS production into the phagosome. However, when the stimulus is very intense, such as after the addition of LPS, IFN-γ or PMA, there was a decrease in ROS liberation, protecting the cells from damage caused by oxidative stress. A summary of reactive products released by macrophages after *Merc sol* treatment is shown in Figure 7.

All the results taken together allow us to conclude that diluted *Merc sol* can alter the immune system *via* macrophages, as these cells play several roles, from wound healing to homeostasis, yet their destructive potential and secretion products, such as ROS, RNS and cytokines, are central to the acute and chronic inflammatory response.

Acknowledgements

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