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The physiology and pharmacology of singlet oxygen

Thomas W. Stief

University Hospital, Marburg, Germany

Summary Reactive oxygen species (ROS) are generated by many different cells. Singlet oxygen ($^1O_2$) and a reaction product of it, excited carbonyls (C=O*), are important ROS. $^1O_2$ and C=O* are nonradicalic and emit light (one photon/molecule) when returning to ground state oxygen. Especially activated polymorphonuclear neutrophil granulocytes (PMN) produce large amounts of $^1O_2$. Via activation of the respiratory burst (NADPH oxidase and myeloperoxidase) they synthesize hypochlorite (NaOCl) and chloramines (in particular *N*-chlorotaurine). Chloramines are selective and stable chemical generators of $^1O_2$. In the human organism, $^1O_2$ is both a signal and a weapon with therapeutic potency against very different pathogens, such as microbes, virus, cancer cells and thrombi. Chloramines at blood concentrations between 1 and 2 mmol/L inactivate lipid enveloped virus and chloramines at blood concentrations below 0.5 mmol/L, i.e. at oxidant concentrations that do not affect thrombocytes or hemostasis factors, act antithrombotically by activation of the physiologic PMN mediated fibrinolysis; this thrombolysis is of selective nature, i.e. it does not impair the hemostasis system of the patient allowing the antithrombotic treatment in patients where the current risky thrombolytic treatment is contraindicated. The action of $^1O_2$ might be compared to the signaling and destroying gunfire of soldiers directed against bandits at night, resulting in an autorecruitment of the physiological inflammatory response. Chloramines (such as the mild and untoxic oxidant chloramine T® (*N*-chloro-*p*-toluene-sulfonamide)) and their signaling and destroying reaction product $^1O_2$ might be promising new therapeutic agents against a multitude of up to now refractory diseases.

INTRODUCTION

The human redox state is a balanced system of pro- and anti-oxidants. The main cellular reactive oxygen species (ROS) are hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen ($^1O_2$). Singlet oxygen – in contrast to the other oxidants – is nonradicalic and excited, i.e. $^1O_2$ or the reaction product of $^1O_2$ with a C=C group, i.e. an excited carbonyl, emits 1 photon when returning to ground state oxygen (1). Whereas the radicalic oxygen species are harmful for the organism, nonradicalic $^1O_2$ is rather mild and untoxic for mammalian tissue. This mild oxidative character has been used for diagnostic purposes, such as the radio-halogenation of proteins (2–4).

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Correspondence to: **Thomas W. Stief**, MD, Priv.-Doz. Department of Clinical Chemistry and Molecular Diagnostics, University Hospital, D-35033 Marburg, Germany. Phone: +49-6421-64471; Fax: +49-6421-65594;
E-mail: thstief@post.med.uni-marburg.de

GENERATION OF $^1O_2$

ROS are generated by pro-oxidative enzyme systems or by redox-cycling of pro-oxidative compounds. Pro-oxidative enzymes are the NADPH-oxidase (5), myeloperoxidase (6), NO-synthase (7,8), or the cytochrome P-450 chain (9–11). Physiologic activation of these pro-oxidative enzymes results into the normal oxidative state. NADPH-oxidase is mainly found in polymorphonuclear leukocytes (PMN). The membranous NADPH-oxidase generates superoxide anions that dismute to hydrogen peroxide. H_2O_2 can react with superoxide anions or with HOCl or chloramines to form the nonradicalic $^1O_2$ (10,11). Since NADPH-oxidase is present in many

different cells (5), diverse cells seem to generate the signal/messenger $^1O_2$ for inter- or intra-cellular signaling.

$^1O_2$ AS A CELL SIGNAL/MESSENGER

$^1O_2$ is a cell signal and messenger (12–14): redox active agents regulate ion channel activity in animals and plants (15). $^1O_2$ activates large-conductance, Ca^{2+}-activated (maxi) K^+ channels (16): monochloramine (NH_2Cl) – in contrast to Tau–Cl – is membrane permeating and at 3–30 μmol/L it increases outward currents more than 8-fold (17,18). $^1O_2$, generated by chloramine-T® (*N*-chloro-*p*-toluene-sulfonamide), also inactivates the Na^+ currents from skeletal or heart muscle fibers, presumably by oxidation of methionine residues (19–21). Chloramine-T® has also been shown to modulate dose dependently outward currents in rabbit atrial cells (22,23) or potassium channels (24–27). Chloramine-T® is known to abolish inactivation of Na^+ and K^+ channels (28–33). Potential receptors for excited oxygen species/light are cryptochromes (34), that consist of flavin- and pteridine- prosthetic groups. Pteridines seem to interact with excited oxygen (35–37).

$^1O_2$ AS A WEAPON

Important armatory functions of $^1O_2$ are:

(a) antiinfectious (antibacterial, antiviral);
(b) cytostatic (anticancer);
(c) antiatherothrombotic (selective thrombolysis).

Ad (a)

Chloramine-T® is bactericidal (38,39). *N*-chloramines exhibit low toxicity and skin irritation and are superior to chlorhexidine in preventing the expansion of the normal skin flora in vivo (40). Chloramine-T® is better than HOCl in inactivation of Staphylococcus aureus (41) and monochloramine is superior to *N*-chlorotaurine in inactivation of Mycobacterium terrae (42). NaOCl shows higher activity than chloramine-T® against Bacillus subtilis spores, coat and cortex material was degraded by chloramine-T® (43).

Because of their untoxicity and antimicrobial power (44), chloramines – especially chloramine-T® – is used for disinfection of drinking water, dialysate, or ice cream machines (45–48). Chloramine T® is also a therapeutic drug for treating bacterial gill disease, a predominant disease of a variety of fish species (49). However, chloramine-T® at 10 g/L (35 mM) has been shown to be ineffective as fungicide (50).

Chloramines are virucidal, too (51–56). Even such dangerous viruses as the Marburg virus (57), or the Ebola virus (58,59) are inactivated by chloramines. Bhanja virus (60), lymphocytic choriomeningitis virus (61), simian rotavirus (62), or poliovirus (63–65) are sensible to NaOCl/chloramines. Even replicating agents of the Creutzfeldt–Jakob disease show some sensibility to NaOCl (74,75).

Poliovirus on whole hands is inactivated (reduction factor >100) by 35 mM chloramine T® (63,67). Coxsackievirus B3, adenovirus type 5, parainfluenza virus type 3 and coronavirus 229E are inactivated (reduction factor >1000) by a 100 mM chloramine-T® solution (68). NaOCl inactivates HIV-1 (66,69–72). The 1.5 mM NaOCl inactivated more than 10 000 fold HIV in serum and 7.5 mM more than 10 fold in blood (73). Own experiments show that chloramine-T® at blood concentrations that are tolerable for normal hemostasis function inactivate the lipid enveloped model virus VSV (vesicular stomatitis virus): 1 mmol/L chloramine-T® inactivates 90% of added VSV, 2 mmol/L chloramine-T® inactivate 99% of added VSV, i.e. there seems to exist a narrow therapeutical window for $^1O_2$ treatment of human infections by enveloped viruses. Intravenous infusions of 1–1.5 mmol/L (blood concentration) chloramine (chloramine-T® or the physiologic *N*-chlorotaurine) once a week for several weeks might be a potent treatment modality for infections with lipid enveloped viruses, such as human immunodeficiency virus (HIV) (74).

Ad (b)

Singlet oxygen is tumoricidal (75). In photodynamic therapy (PDT) high concentrations of singlet oxygen are generated by illumination of a photosensitizer, resulting in a cytostatic action of PDT (76,77). However, excessive oxidant concentrations are carcinogenic (78–82).

Ad (c)

$^1O_2$ mediates PMN adherence to the endothelium (12,83,84) and subsequently selective thrombolysis (10,11). $^1O_2$ activates the complement cascade, transforming C5 into a C5b-like molecule (85); activation of the complement cascade results in increased PMN adhesion to endothelial cells (86,87). Since cholesterol is an inhibitor of $^1O_2$, the atherogenic action of cholesterol might be explained by insufficient thrombolytic capacity of a hypercholesterolemic individuum (10,11,88).

TOXICOLOGY OF $^1O_2$

However, and according to Paracelsus (dosis sola venenum facit (only the dosage makes the poison)), high concentrations of chloramines can act toxic to normal tissue (89). 3 mM monochloramine induced DNA breakage (90). PMN are the main cells that use singlet oxygen as

a weapon. They also dispose of an enzyme that reverses methionine oxidation – the methionine sulfoxide-peptide reductase (91). Taurine–chloramine is the major chloramine generated in activated PMN as a result of the reaction between HOCl (92) and taurine, an abundant free amino acid in their cytosol (93–96). Also other plasma proteins react with hypochlorite to chloramines (97). HOCl (25 μM) or NH_2Cl (10 μM) – but not Tau–Cl (100 μM) – increase endothelial permeability (98) or epithelial cell injury (99). NH_2Cl, the reaction product of hypochlorite with ammonia (NH_3), seems to be more toxic than Tau–Cl (100,101). The 60 mM NH_2Cl (about 10 times the concentration generated by activated PMN!) is ulcerogenic in rat stomachs, taurine application (1 ml 200 mM) attenuates the deleterious action of NH_2Cl (102), NH_2Cl induces apoptosis in gastric mucosa (103). Tau–Cl selectively modulates the ability of dendritic cells to induce the release of IL-2 and IL-10 from T cells (104). Tau–Cl inhibits monocyte chemoattractant protein-1 and macrophage inflammatory protein-2 production in glioma cells (105). Tau–Cl inhibits the production of NO and superoxide anions (106–109), prostaglandin E2 (110, 111), interleukin 6, and tumor necrosis factor-α and it has been suggested that Tau–Cl may regulate the balance between protective, microbicidal and toxic effect of PMN, Tau–Cl at 0.1–0.3 mM inhibits interleukin-2 release of purified T cells (112).

Chloramines – in contrast to sodium chlorite – do not induce detectable hematologic (→ methemogloblin) or hepatic (→ elevation of serum alanine-amino-transferase) in African Green monkeys (113). However, a chloramine-induced haemolysis and erythropoietin resistance occurred when the dialysate chloramine levels rose from <0.1 to 0.3 p.p.m. (about 1 mM) resulting in an increase in mean methaemoglobin of 23% and a 21% fall in mean haptoglobin during haemodialysis; only one patient with glucose-6-phosphate-dehydrogenase deficiency had Heinz bodies (114,115). Dogs treated with 1 mmol/L blood concentration of chloramine T® 3 times a week for several months did not show toxic side effects (116).

CONCLUSION

Singlet oxygen is a major agent generated by many different cell types, especially by neutrophil granulocytes. $^1O_2$ is nonradicalic and emits light when returning to ground state oxygen. Like the gunfire of soldiers directed against bandits, $^1O_2$ is both a signal and a weapon, directed against multiple pathogens – including microbes, virus, cancer cells, thrombi – and resulting in an autorecruitment of the physiological inflammatory response. Chloramines are stable chemical generators of $^1O_2$. *N*-chlorotaurine is an important physiological chloramine, for therapeutic purposes chloramine-T® seems to be a promising new therapeutic agent against a multitude of up to now refractory diseases.

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