Current Trends in Microbiology
Vol. 13, 2019

Mini-Review

‘Less is more’: transferring a principle from art to science

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ABSTRACT

‘Less is more’ is a common saying that can be applied for instance in arts. In architecture, this was typical for the Bauhaus team whose design was inspired by this principle, incarnated by Mies van der Rohe in his buildings. It may be surprising at the first moment that the principle can be applied in research and development, too. We here present the example N-chlorotaurine, an antiseptic and anti-infective derived from the human defence system, whose low reactivity (‘less’) is the reason for its broader applicability and remarkably also for better efficiency (‘more’). N-chlorotaurine belongs to the long-lived oxidants produced by activated human granulocytes and monocytes. It can also be prepared synthetically and used topically as an anti-infective. Its oxidizing and chlorinating activity is lower than that of other chloramines. Therefore, its tolerability is higher, and it can be applied to different body sites, including sensitive ones such as ulcerated skin, the eye, the ear, the urinary bladder, organ abscesses, and the bronchopulmonary system. N-chlorotaurine can be applied in higher concentration, which allows to easily overcome chlorine consumption. Moreover, its activity is enhanced by transfer of its active chlorine to ammonium in human exudates and body fluids, forming the more lipophilic and higher microbicidal monochloramine. Therefore, it is so far the only antiseptic whose microbicidal activity is not decreased but enhanced under *in-vivo* conditions. These findings are confirmed by the curative effects of N-chlorotaurine in clinical studies and case applications, and thus the principle ‘less is more’ applies to this antiseptic and anti-infective substance and renders it promising for further development.

KEYWORDS: chloramines, antiseptic, N-chlorotaurine, hypochlorite, anti-infective, tolerability.

INTRODUCTION

Disinfectants and antiseptics are widely used in medicine to inactivate virulent pathogens and to avoid infection. Generally, disinfectants with strong microbicidal activity are desired since surfaces should be cleared of infectious agents rapidly. The same principle is basically valid for antiseptics, too, which are used on the human skin or mucous membranes. As the example known best, substances used for hand disinfection must have a strong and rapid microbicidal activity. In addition, in present times of increasing resistance against antibiotics, antiseptics are a meaningful and advantageous alternative in topical treatment of infections of different body sites. In the presence of body fluids and exudates, however, antiseptics undergo chemical reactions with organic matter, which reduces their concentration and antimicrobial activity and decreases their efficacy [1-3]. Particularly with active halogen compounds, this reduction of activity increases with their reactivity [2]. Investigations disclosed that active chlorine representatives with low reactivity overcome this problem due to two facts. First, they are better tolerated and can be applied at higher concentrations [4]. Second, they undergo transchlorination reactions at a higher rate, which may even enhance their activity in the presence of

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organic load [1]. These facts led to the development of a new concept that active chlorine compounds with low reactivity are more advantageous compared to those with high reactivity, for topical treatment of infections. This concept is explained in the following sections.

Approach to 'less is more'

Referring to testimonies in the field of arts like poetry, painting and architecture, the saying 'less is more' insinuates the recommendation to ignore nonessential details, which is a basic challenge for making art works unique. As a characteristic artist who was very fond and convinced of 'less is more' one can cite Mies van der Rohe, the preeminent architect of the Bauhaus team whose concept of design was intensively inspired by this principle. The purpose of this paper is to record the fact that 'less is more' is true also in the field of science, for example experimental hygiene, albeit in an other context.

As an aid for understanding this amazing message, considering the problem of 'irritation versus bactericidal activity in disinfection of living tissue with oxidants' might help. Irritation means impairing the chemical structure of the skin surface, which has the consequence of several unwanted adverse effects like injury of cell membranes, tissue inflammation, penetration and consumption of agent, and in the end also sense of pain. Bactericidal activity, on the other hand, refers to disintegration of enzymes and structures essential to life of bacteria.

The extent of both manifestations, caused by the chosen disinfecting agent, varies with its oxidizing potency. Generally, stronger irritation takes place with a stronger agent (which exerts a comparatively high oxidizing potency, e.g. hypochlorous acid, HOCl) compared with a weaker one, which is sufficient for killing of bacteria, too (e.g. N-chlorotaurine, NCT) [5].

While the problem of irritation with a too reactive agent can be mastered using a lower concentration, its antimicrobial activity may become too low.

There are two possibilities to cope with such a situation. First, the infected area can be flushed intensively with a very diluted solution of the strong oxidant (e.g. HOCl). The irritating potency is reduced by the low concentration, but the disadvantage is that application to achieve sufficient bactericidal action is not easy. Therefore, it should be replaced with the second option. Since irritation is caused by agents that are more aggressive, choosing an agent with lower activity at a higher concentration can solve the problem.

In this view, we can reformulate the title 'Less is more' into 'Less activity is more efficiency.'

Multiple experiments in this field have revealed overwhelmingly that the body-own antiseptic N-chlorotaurine (NCT, Cl-HN-CH_2-CH_2-SO_3Na**)** turned out as the agent of choice securing both adequate bactericidal activity and low irritation.

In the following paragraphs, several examples are presented that confirm this assertion.

Enhancement of activity of NCT by amino compounds

Due to the mild oxidizing activity of NCT, its microbicidal activity also is low compared to strong oxidants like hypochlorous acid. In the presence of amino compounds, however, the chlorine consumption of NCT is significantly lower than that of strong active chlorine compounds such that the maintained oxidation capacity is higher [2]. According to this fact, NCT and other chloramines (R-NHCl compounds) formed *in vivo* during inflammation from the reaction of HOCl with amino groups have been designated as 'long-lived oxidants' [6, 7]. A consequence of the low reactivity is that transchlorination reactions (transfer of active chlorine to amino compounds nearby in equilibrium) become relevant [6, 8].

$$R^1\text{-NHCl} + R^2\text{-NH}_2 \leftrightarrow R^1\text{-NH}_2 + R^2\text{-NHCl}$$

Oxidation capacity is maintained during these transchlorination reactions. In general, the microbicidal activity of active chlorine compounds is decreased by transchlorination since the activity of the formed chloramines is lower, in particular if their molecular weight is high [9]. For NCT, however, whose reactivity is on the low end, the situation turned out different. While addition of albumin expectedly decreased its bactericidal activity, it was significantly enhanced in the presence of the low molecular weight molecules

glycine and alanine due to the formation of the respective N-chloro amino acids [9]. The so far largest effect was found with NCT plus ammonium chloride by formation of monochloramine (NH_2Cl) [6, 10-12].

$Cl\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}SO_3^- + NH_4Cl \leftrightarrow H_2N\text{-}CH_2\text{-}CH_2\text{-}SO_3^- + NH_2Cl + H^+ + Cl^-$

The stronger bactericidal and particularly fungicidal activity of monochloramine is due to its higher lipophilicity and small molecular weight and bulk, which both relieve penetration into the microbes, being an essential cause for killing [6, 10-12]. Accordingly, in buffer solutions the bactericidal efficacy of NCT was significantly higher in the presence of NH_4Cl [13], and the effect was even more pronounced with fungi [13-17]. Even mycobacteria and parasites including their cystic forms were killed by NCT plus NH_4Cl [18-22]. In view of practical application of NCT as an anti-infective, enhancement of its activity against bacteria and fungi was demonstrated in exudates from different body sites [9], nasal secretion [16], artificial sputum medium [23], urine [24], and plasma [25], while the activity was decreased in whole blood because of the high density of antioxidants (e.g. glutathione) [26].

A comparison of the microbicidal activity of NCT with strong active chlorine and bromine compounds under protein load impressively disclosed that the weak oxidant can outmatch the strong one [1]. An example is provided in Figure 1 showing the bactericidal activity of NCT and the highly reactive NaOCl in buffer solution and 5% peptone. While the activity of NCT is enhanced by more than 2-fold ($P < 0.01$), that of NaOCl is decreased by more than 1000-fold ($P < 0.01$).

Efficacy of NCT in clinical studies and single applications

The results mentioned in the previous section contribute in explaining the efficacy of NCT upon clinical application in humans. In a phase II clinical study in purulently coated crural ulcerations, 1% NCT was not only better tolerated, as expected, but also showed a similar anti-infective activity compared to the markedly stronger oxidant chloramine T [27]. Application of NCT strips in external otitis led to a significantly more rapid healing than the standard therapy with topical antibiotics and corticoids [28]. For treatment of conjunctivitis, NCT was shown to be effective against bacteria, viruses, and fungi in clinical studies and cases [15, 29, 30]. *In-situ* studies in cornea infection models elucidated that addition of NH_4Cl to NCT enhances the penetration of active chlorine and inactivates bacteria, fungi, and acanthamoebae more rapidly due to the formation of NH_2Cl [31, 32]. This was confirmed with adenoviruses in the rabbit model [33]. Since a concentration of 0.1% NCT plus 0.1% NH_4Cl, which exerts strong microbicidal activity, was well tolerated as eyedrops in humans [34], it is of high interest for treatment of particularly fungal, protozoal and viral keratoconjunctivitis. In body fluids containing a high concentration of ammonium, plain NCT has a very good microbicidal activity, for instance in urinary tract infections where it may be considered as an irrigation solution against antibiotic-resistant pathogens [24, 35]. Experimental gonarthritis by *Staphylococcus aureus* in mice was mitigated by topical NCT [36]. Recent case reports confirm the efficacy of NCT in skin infections, such as herpes zoster and purulent bacterial infections [37, 38].

Tolerability of NCT

In 1971, NCT was first discovered as an indirect product of myeloperoxidase of human granulocytes [39], generated by the reaction of HOCl with the abundant taurine [6]. Since downregulation of pro-inflammatory cytokines and chemokines was found at micromolar physiological concentrations, anti-inflammatory effects and contribution to termination of inflammation are assumed to be main functions of NCT *in vivo* [40-42]. Additional investigations clearly demonstrated that toxic effects of HOCl are attenuated or removed in the presence of excess of taurine, whereby the whole active chlorine is transferred to NCT [43-47]. Because of the reaction of HOCl with taurine to produce NCT, particularly at alkaline pH, where the formation of NCT exceeds that of N-dichlorotaurine, taurine protected lung epithelial cells from HOCl-induced injury [48]. A similar protective effect from HOCl was seen with taurine in canine erythrocytes *in vitro* and in the albino rabbit eye *in vivo* [49].

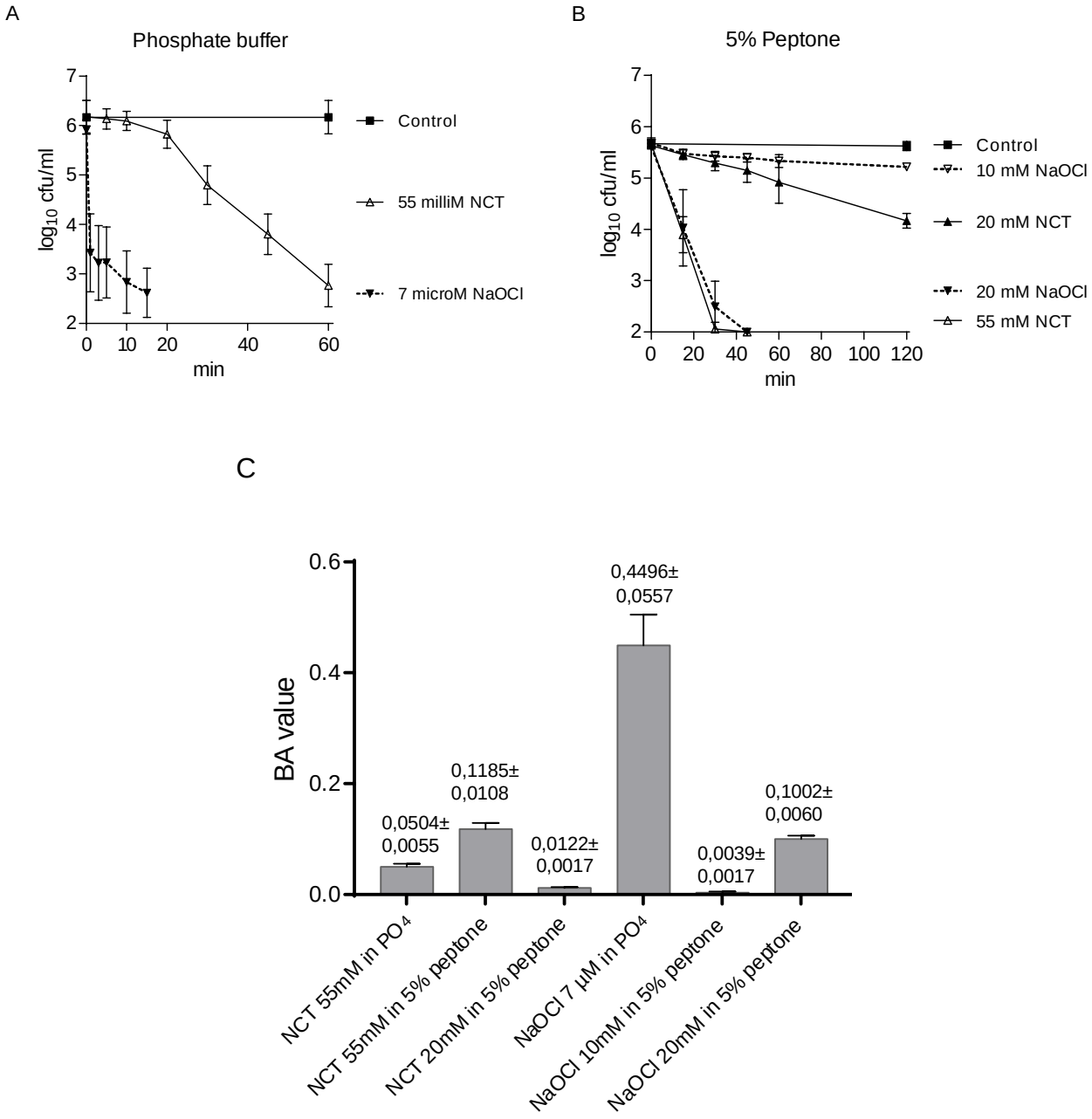

Figure 1. Bactericidal activity of NaOCl and NCT against *S. aureus* ATCC 25923 at RT and pH 7.0-7.1 in plain 0.1 M phosphate buffer solution (A) or in 5% peptone enzymatic digest from casein (Fluka, lot 0001437645 100988706, rich in amino acids and peptides, ammonia test positive) dissolved in phosphate buffer (B). Mean values ± standard deviation of three to five independent experiments. $P < 0.01$ between phosphate buffer and peptone for both NaOCl and NCT (one-way ANOVA and Tukey's multiple comparison test). NaOCl (Lactan® GmbH, Graz, Austria, lot 509291486 Carl Roth®); NCT (produced by M. Nagl and W. Gottardi, lot 2019-09-24). Controls in buffer and 5% peptone, respectively, without antiseptics. Quantitative killing assays, detection limit 2 log10 colony-forming units (cfu)/ml. (C) 'Bactericidal Acivity' values (BA values) calculated with the Integral Method [52], which condenses the whole killing curve into one value, are shown, too. The higher the value, the higher the bactericidal activity. Mind the enormous decrease of activity of NaOCl in peptone by > 1000-fold taking into account concentration and BA value, which is in strong contrast to 1% NCT (55 mM) whose activity is significantly increased in 5% peptone ($P < 0.01$, Student's unpaired t test).

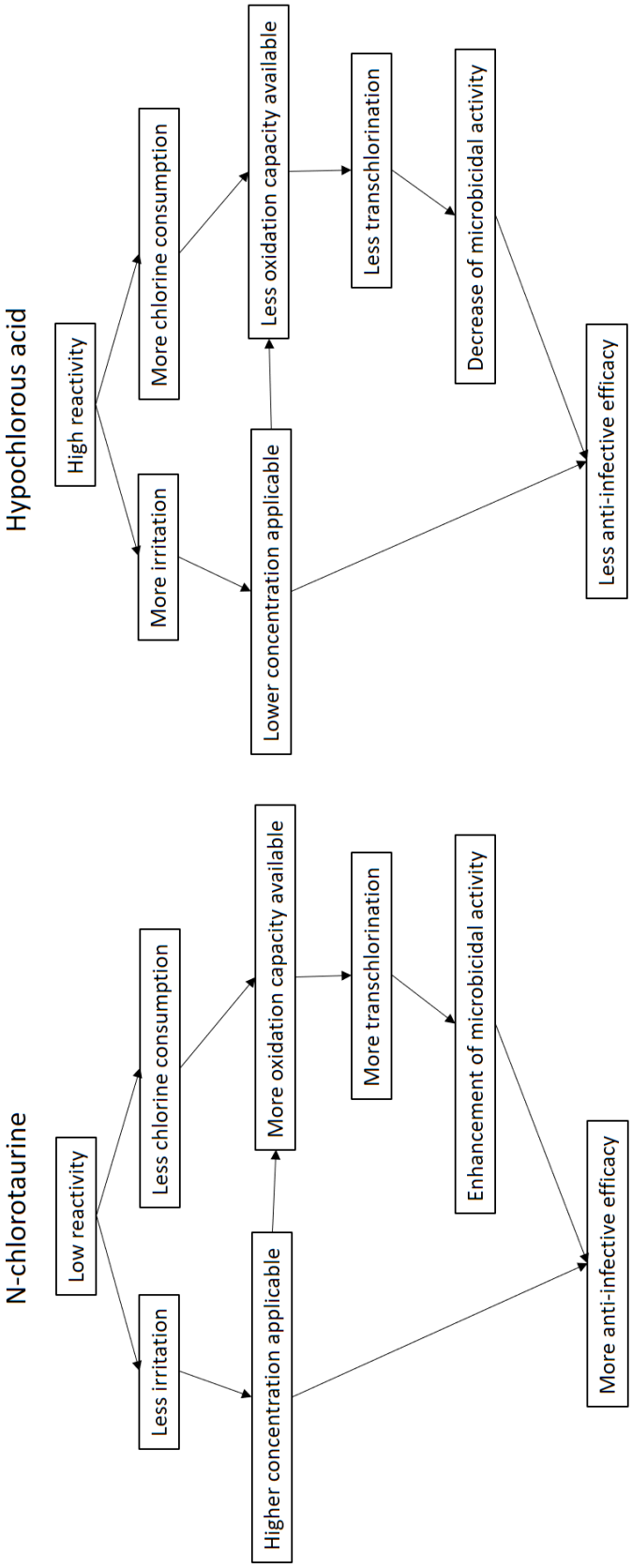

Figure 2. Comparison of an active halogen compound with low reactivity (e.g. N-chlorotaurine) and high reactivity (e.g. hypochlorous acid) in the presence of amino compounds, for instance body fluids and exudates.

HOCl (2.7 µM) markedly increased the perfusion pressure and the release of lactate dehydrogenase associated with a decrease in bile flow, while these effects were only slightly expressed by 65 µM NCT [50]. HOCl or NH_2Cl, but not NCT, caused a reversible shortening of the cytoskeletal actin microfilaments, cell retraction, and increased permeability of cultured bovine aorta endothelial monolayers and rapidly increased microvascular permeability in isolated perfused rat lungs [51].

Moreover, in all of the clinical studies and case applications performed so far (see previous section), the only adverse effects of NCT were a transient local itching and burning. No toxic topical or any systemic effects occurred, for review see [4, 5, 15]. The direct comparison with the higher reactive active chlorine compound chloramine T (CAT) in crural ulcerations disclosed less pain and earlier granulation and re-epithelialization in the NCT group [27]. Even inhalation of 1% NCT is safe according to a recent phase I trial [52], which opens the window for further investigations in suggestive indications such as chronic obstructive pulmonary disease and cystic fibrosis. The good tolerability of NCT can be explained clearly by its low oxidizing reactivity.

Synopsis of properties of NCT

As a mild oxidant, the chemical reactivity of NCT is limited to mainly thio and amino groups [53]. Therefore and because of its hydrophilicity (ionic compound), its cytotoxicity is lower than that of all other presently used active halogen compounds [5]. This and its endogenous nature explain its high tolerability [4]. Accordingly, the microbicidal activity of NCT is markedly lower than that of higher reactive chloramines [5]. In the presence of organic material such as exudate or body fluids, however, its chlorine consumption is lower and transchlorination enhances its microbicidal activity much more than its irritating one. Moreover, NCT can be applied in a high concentration of 1%, which would be toxic with other active chlorine compounds, but warrants sufficient oxidation capacity even in the presence of higher protein load. Under these circumstances, not only the tolerability, but also the microbicidal efficacy of NCT can outmatch higher reactive representatives of this class of antiseptics. Clinical results confirm these findings.

Therefore, treatment of infections with a low reactive chloramine turns out to be advantageous over a highly reactive one, particularly at sensitive body sites or in the presence of high organic load – exactly in terms of ‘less is more’ or ‘less reactivity is more efficiency’. These considerations are summarized in Figure 2.

Final remark

Nearly 30 years ago, when the authors were successful in synthesizing the body-own antiseptic N-chlorotaurine for which a patent was granted, the innovation was presented to notable pharmaceutical companies. The response was very modest. Only two companies (Johnson & Johnson from USA and Taisho from Japan) requested a sample for testing. Both basically responded in the same way: N-chlorotaurine lacked sufficient efficiency as an agent in biological systems. In both cases, however, it remained unclear, which biological activity was regarded. Johnson & Johnson specified their message by the information that new consigned substances are considered worth for a substantial examination only if they exhibit a biological activity at a concentration of 0.001%. Since N-chlorotaurine accomplished this threshold only at a concentration of 0.01%, it was not further investigated and sent back.

This small account points to the fact that in days gone by, the real meanings of the issues bactericidal activity and irritation as well as their intrinsic context have not been fully understood.

ACKNOWLEDGEMENTS

We are grateful to Andrea Windisch for excellent technical assistance.

FUNDING

There was no separate funding for this publication.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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