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Research Article

Assessment of Damage to Nucleic Acids and Repair Machinery in *Salmonella typhimurium* Exposed to Chlorine

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Water disinfection is usually evaluated using mandatory methods based on cell culturability. However, such methods do not consider the potential of cells to recover, which should also be kept as low as possible. In this paper, we hypothesized that a successful disinfection is achieved only when the applied chlorine leads to both intracellular nucleic acid damage and strong alterations of the DNA repair machinery. Monitoring the SOS system responsiveness with a *umuC*'-'*lacZ* reporter fusion, we found that the expression of this important cellular machinery was altered after the beginning of membrane permeabilization but prior to the total decline of both the cell culturability and the nucleic acid integrity as revealed by Sybr-II staining. Rapid measurement of such nucleic acid alterations by fluorochrome-based staining could be used as an alternative method for assessing the effectiveness of disinfection with chlorine.

1. Introduction

Chlorine (a mixture of HClO and ClO^-) is the most widely used bactericidal agent for disinfection of drinking waters. Chlorine reacts with various biological molecules: proteins [1–3], lipids [4, 5], and nucleic acids [6–9]. By way of consequence, this strong oxidant affects structures and several metabolic processes such as membrane permeability [10–12], ATPase activity [13, 14], respiration [11], and the proton motive force of the cell [15]. All these deleterious effects were previously shown to occur very rapidly [16, 17].

One of the problems related to water disinfection with chlorine is linked to the control of the effectiveness of disinfection, which requires carrying out mandatory methods such as culturing bacteria on standard nutritive agar media. These mandatory methods give delayed results and, additionally, do underestimate the real number of viable bacteria in drinking water, especially when oxidative stress has been applied [11, 18, 19]. Then, the question of an optimal and effective dose of disinfectant (the dose which should prevent the repair of injured cells and their regrowth) has been left unanswered both (i) because the key functions or structures to be irreversibly targeted by the disinfection process have not been defined yet, and (ii) because there is no accurate and rapid method currently available for detecting irreversible injuries to be used as an indicator of treatment effectiveness.

Reactivity of HClO at lethal concentrations with nucleic acids is governed by chlorine diffusion into the cells and its direct action on cell polymers as well as by reactive oxygen species generated upon exposure to the oxidant [17, 20, 21]. Moreover, chlorine attacks preferentially exocyclic-NH2 groups of cytidine and adenosine at specific sites [8] and may also lead to DNA backbone cleavage [20, 22]. Saby et al. [23] first showed that chlorine-induced damage to nucleic acids could be revealed by the inability of fluorochromes, such as DAPI, to stain chlorinated bacteria. Other studies have corroborated this result and showed that chlorine reacting with nucleic acids in vitro and in vivo caused damage, thus resulting in a reduced fluorescence of the complex (nucleic acid + fluorochromes) stained with SYBR-II or propidium iodide (PI) [12, 24, 25].

A rapid analytical method which could confirm the irreversible and growth inhibitory nature of the damage suffered by chlorinated cells would clearly help practitioners