Published in final edited form as:

Trends Microbiol. 2017 August ; 25(8): 602–603. doi:10.1016/j.tim.2017.05.009.

Effect of CO₂ on Peroxynitrite-mediated Bacteria Killing: Response to Tsikas *et al*

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Keywords

Helicobacter pylori; peroxynitrite; nitrosoperoxycarbonate

We appreciate that our Review Article on the crosstalk between host nitric oxide (NO) and the gastric pathogen *Helicobacter pylori* [1] has stimulated debate about the potential role of CO₂ in peroxynitrite (ONOO⁻)-mediated toxicity toward the bacterium. This topic has strong biological relevance since *H. pylori* uses its urease to convert urea into CO₂ and ammonia, the last molecule being involved in the resistance to the acidic conditions encountered by the bacterium in the stomach. Although there is a consensus to stipulate that CO₂ and ONOO⁻ react to form nitrosoperoxycarbonate anion (ONOOCO₂⁻) [2], the protective or deleterious effect of this product remains a subject of controversy.

In this context, we reported in our review the work by Kuwahara *et al.* showing that CO₂ inhibits the bacteriotoxic effect of ONOO⁻ [3]. These authors demonstrated that *i*) the ONOO⁻-dependent killing of wild-type *H. pylori*, but not of a urease-deficient strain, was attenuated by adding urea to the culture medium, and the protection from killing was also observed with addition of recombinant urease; *ii*) the protective effect of urea on ONOO⁻-mediated *H. pylori* killing was reversed by either of two urease inhibitors; *iii*) NaHCO₃, but not NH₃, inhibited the bactericidal effect of ONOO⁻; and *iv*) the amount of nitrotyrosine in *H. pylori* exposed to ONOO⁻ was dramatically inhibited by adding urea or NaHCO₃ [3]. In our opinion, this study clearly demonstrates that CO₂ synthesized by the activity of urease dampens the killing effect of ONOO⁻ on *H. pylori*.

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Conversely, in their letter, Tsikas *et al.* suggest that CO₂ could theoretically lead to increased ONOO⁻-dependent oxidative damage in *H. pylori*. This postulate is first based on chemical evidence that ONOO⁻ oxidant properties are increased by physiological concentrations of CO₂. To evaluate this argument we examined the original literature, which indicates that HCO₃⁻ or CO₂ potentiate the ONOO⁻-mediated nitration of aromatic compounds [2, 4]; however this has not been demonstrated to be involved *H. pylori* killing to our knowledge. However it is known that *S*-nitrosylation is involved in the antibacterial effect of NO, by targeting essential *H. pylori* proteins such as UreA or GroEL [5]. Because CO₂ can reduce the level of *S*-nitrosylation [2], this provides an additional chemical mechanism through which urease activity and the generation of CO₂ can potentiate the survival of *H. pylori*, rather than impairing it. Moreover, the theory raised by Tsikas *et al.* that signaling in *H. pylori* requires *S*-nitrosothiols, and could be therefore inhibited by CO₂, is highly speculative, since this phenomenon is extremely limited in the bacterial kingdom [6, 7].

Tsikas *et al.* also suggest that ONOOCO₂⁻ homolysis yields the generation of the more toxic radical species, NO₂[•] and CO₃^{•-} [8, 9]. However, this reaction has been noted to occur with only 30–40% of the ONOOCO₂⁻ [8, 9]. Rather, it has been suggested that the most probable reaction of ONOOCO₂⁻ in aqueous medium is hydrolysis, producing the nontoxic molecules carbonate and nitrate [10]. Finally, Tsikas *et al.* speculate that if CO₂ were protective, it would occur independently from formation of ONOOCO₂⁻ and instead by generation of bicarbonate through the activity of carbonic anhydrases, thus regulating pH. However, it is unlikely that carbonic anhydrases play a role in protection against ONOO⁻, because Kuwahara *et al.* have shown that *i*) pH was not affected in the culture of *H. pylori* treated with ONOO⁻ and/or CO₂ and *ii*) adding exogenous NH₃ did not affect ONOO⁻-dependent bacterial killing [3]. An alternative concept that one could imagine would be that endogenous CO₂ could induce an NO/ONOO⁻ detoxification system such as peroxiredoxin [11], but this remains unknown.

While we take note of the arguments developed by Tsikas *et al.*, we caution that conclusions about biological significance should not be directly drawn from chemical reductionist models in which highly purified compounds interact. This is particularly true for the present case, mainly because Tsikas *et al.* did not envision in their scheme that urease-derived CO₂ may interact with ONOO⁻ outside the bacteria, as mentioned by Kuwahara *et al.* in their discussion [3]. This would also explain why Kuwahara *et al.* observed that urea treatment decreases intracellular nitrotyrosine in *H. pylori* exposed to ONOO⁻, although it is well known that CO₂ favors ONOO⁻-mediated nitration [2, 4]. We suggest that experiments analyzing nitrotyrosine in *H. pylori* supernatants would help in the delineation of this discrepancy. Another suggestion would be the assessment of effects of urease in other urease-positive bacteria on resistance to nitrosative stress, particularly because such organisms can constitute a substantial part of the human gastric microbiome [12].

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