doi:10.1246/cl.2010.322
322 Published on the web February 27, 2010

Methylglyoxal: A New Weapon against Staphylococcal Wound Infections?

Marco Fidaleo,[1] Antonio Zuorro,[2] and Roberto Lavecchia*[2]
[1]*Department of Cellular and Developmental Biology, "La Sapienza" University, P.le Aldo Moro 5, 00185 Rome, Italy*
[2]*Department of Chemical Engineering, "La Sapienza" University, Via Eudossiana 18, 00184 Rome, Italy*

(Received December 4, 2009; CL-091075; E-mail: roberto.lavecchia@uniroma1.it)

Methylglyoxal (MG), a phytochemical present in some New Zealand honeys, was found to possess bactericidal activity against *S. aureus* and a methicillin-resistant strain of *S. epidermidis*. The MIC and MBC values were 1.05 and 2.11 mM, respectively. Inclusion of MG in a hydrogel resulted in an active and stable preparation suitable for treating wound or burn infections.

The origin of the unusually high antimicrobial activity of Manuka honey, the honey derived from the native New Zealand manuka tree (*Leptospermum scoparium*), has been the subject of intense research over the last few decades.[1] Recently, strong evidence has been provided that methylglyoxal (MG), a phytochemical found in the nectar of manuka flowers, could be the main responsible for its antimicrobial properties.[2] Prompted by these results and considering the well-documented efficacy of Manuka honey in wound healing,[3] we decided to investigate the antibacterial potency of MG, in liquid and gel formulations, against the pathogens most frequently associated with wound infections. As a first step to this end, we focused our attention on the two major opportunistic pathogens of the genus *Staphylococcus*: *S. aureus* and *S. epidermidis*, because of their increasing prevalence in nosocomial and community-acquired infections.[4] Treatment of these infections is often difficult due to the emergence of resistance to most first-line antimicrobial agents, such as penicillins, tetracycline, chloramphenicol, and macrolides.

S. aureus strain ATCC 25923 was obtained from KairoSafe (Duino Aurisina, Italy), while *S. epidermidis* was isolated at the Department of Cardiac Surgery ("Tor Vergata" University, Rome, Italy) from the wound site of a patient who developed infection after heart surgery. MG, Mueller–Hinton Agar 2 and Mueller–Hinton Broth were from Sigma-Aldrich (Milano, Italy). All other chemicals were of analytical grade and used without further purification.

The MG hydrogel formulation (at 0.5% w/w of the active substance) was prepared by conventional pharmaceutical techniques[5] using Carbopol® 940 as the gelling agent and the appropriate excipients.

MG susceptibility tests were performed by the agar-well diffusion methods. Briefly, bacterial strains from an exponential-phase culture, obtained from a single colony, were spread on the surface of agar plates using a sterile swab soaked in the bacterial suspension. 9-mm wells were then cut in the agar and filled with 150 µL of an aqueous solution at the appropriate MG concentration. After overnight incubation at 37 °C, the plates were examined and the diameters of the inhibition zones were measured. MIC and MBC values were determined in Mueller–Hinton Broth using a twofold serial dilution technique. Bacterial strains from an exponential-phase culture were grown overnight at 37 °C, using an inoculum of approximately 1.5×10^6 CFU mL^{-1} and different concentrations of MG. Subcultures were then streaked on Mueller–Hinton Agar plates, which were incubated at 37 °C for 18 h. After this time, the number of colonies formed on each plate was counted. The MIC was defined as the lowest concentration of MG giving complete inhibition of bacterial growth, and the MBC as the lowest concentration killing 99.9% of the original inoculum. All experiments were done at least in triplicate and the results were averaged.

To assess the long-term stability of the antimicrobial gel formulation, thermally accelerated aging experiments were also made. In these runs the MG-containing hydrogel was subjected to heating between 40 and 100 °C for up to 144 h. After treatment, the gel was cooled to room temperature and the antimicrobial activity was determined as described above.

Preliminary testing of bacterial susceptibility to common antibiotics indicated that the strain isolated from patient's wound was a methicillin-resistant *S. epidermidis* (MRSE). This is not surprising if one considers that about 80% of *S. epidermidis* from nosocomial infections are resistant to methicillin.[6]

Typical zones of inhibition produced by MG (in liquid or gel formulations) against MRSE are shown in Figure 1. Agar-well diffusion tests gave the results presented in Figure 2. As evident, both species were highly sensitivity to MG and exhibited a dose-dependent response.

Susceptibilities to MG were very similar for the two pathogens, with average inhibition zone diameters ranging from 18 to 40 mm as MG concentration was increased from 1.25 to 15 mg mL^{-1}. Such similarity was also confirmed by the identical MIC and MBC values, which were 1.05 and 2.11 mM, respectively.

MG is a highly reactive α-ketoaldehyde which is normally produced in living organisms through enzymatic and non-enzymatic pathways, including protein glycation by glucose, lipid peroxidation, and the metabolism of acetone and threonine.[7]

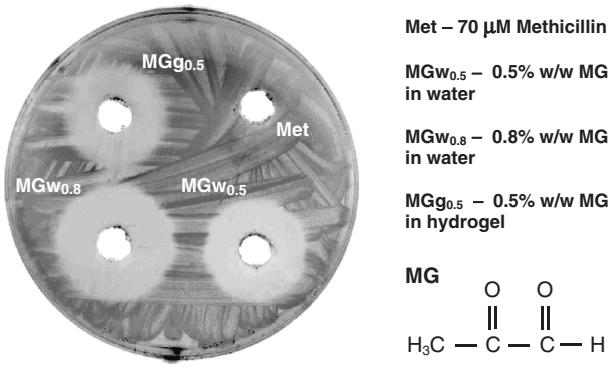

Figure 1. Inhibition zones produced by methicillin (Met) and by MG (in solution or in hydrogel) against MRSE.

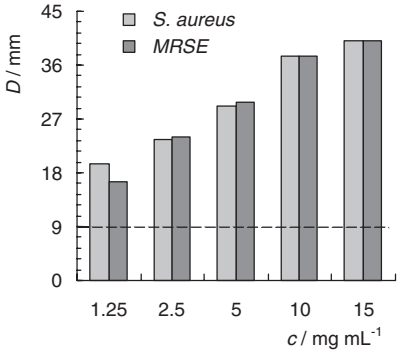

Figure 2. Effect of MG concentration (c) on the mean diameter of inhibition (*D*) for *S. aureus* and MRSE. The dashed line indicates the size of the agar well.

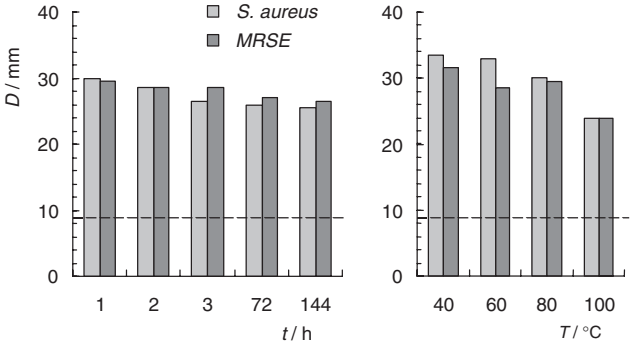

Figure 3. Effect of heating on the activity of the hydrogel: up to 144 h at 80 °C (left) and 1 h at various temperatures (right). The dashed lines indicate the size of the agar well, and *D* is the mean diameter of inhibition.

Table 1. Mean diameters of inhibition (mm) for *S. aureus* and MRSE in the presence of 0.5% w/w MG in solution or in hydrogel

Microorganism	MG in solution	MG in hydrogel
S. aureus	29.75 ± 0.96	29.64 ± 1.59
MRSE	29.25 ± 3.20	29.07 ± 1.35

MG is characterized by a small molecular size ($M_r = 72.06$ Da), a high hydrophilicity, and the presence of both an aldehyde and a ketone group in the molecule (Figure 1). All these factors are most probably involved in its biocidal action, allowing fast diffusion of the molecule across the outer membrane and the periplasmic space of microbial cells, and effective interaction with target sites. Regarding the nature of these sites, it should be considered that MG, like other 2-oxoaldehydes, reacts readily with thiol groups of proteins as well as with guanine bases of DNA, leading to inhibition of some enzyme activities and causing arrest of cell division.[8] It can, therefore, be speculated that its inhibitory and bactericidal activity are the result of an overall cellular damage caused by random multiple detrimental effects on cytoplasmic constituents rather than interaction with specific target sites.[9]

As can be seen from Table 1, no reduction in activity was observed when MG was incorporated (at 0.5% w/w) in the hydrogel. The average inhibition zone size for the staphylococcal species was 29.3 ± 0.4 mm, using the gel, and 29.5 ± 0.3 mm with MG in solution. Aging experiments showed that the antimicrobial properties of MG in the gel were largely preserved after heating (Figure 3). Exposure to 80 °C for up to 144 h resulted in only small changes in activity, with an average inhibition zone diameter decreasing from 29.7 ± 0.4 to 26 ± 0.7 mm. Even under the most severe conditions, 1 h heating at 100 °C, the effects on antimicrobial activity were fairly mild, being more than 75% of the MG activity maintained. Thus, it can be deduced that stable topical formulations can be easily obtained by incorporation of MG in a gel of the type considered here.

Assessment of possible cytotoxicity of MG is, of course, of paramount importance. In this connection, it should be pointed out that long-term toxicity and pharmacokinetic studies performed on different species of animals (mice, rats, rabbits, and dogs) indicated that MG is potentially safe for human consumption.[10] The same conclusions can be drawn from a recent review on the potential toxic effects of MG in vivo.[11] Moreover, focusing attention on the proposed use of MG, i.e., in topical applications, it is also worth noting that hydroxycarbonyl compounds such as dihydroxyacetone, glyceraldehydes, and MG are already utilized in cosmetic preparations and are generally recognized as safe for such uses.[12]

In conclusion, the results from the present study indicate that liquid and gel formulations containing MG have high bactericidal activity against staphylococcal species, including the methicillin-resistant *S. epidermidis* which is becoming an increasingly frequent cause of nosocomial infections. We, therefore, recommend that further research be undertaken to explore in depth the potential of MG as a new option for the treatment of wounds infected or at risk of infection with microbial pathogens.

We wish to thank Prof. Antonio Scafuri (Department of Cardiac Surgery, "Tor Vergata" University, Rome, Italy) for his contribution and valuable discussion. We are also grateful to Prof. Claudia Sartori and Prof. Paola Ballario ("La Sapienza" University, Rome, Italy) for logistic support.

References

1 M. J. Snow, M. Manley-Harris, *Food Chem.* **2004**, *84*, 145; P. E. Lusby, A. L. Coombes, J. M. Wilkinson, *Arch. Med. Res.* **2005**, *36*, 464; S. E. Blair, N. N. Cokcetin, E. J. Harry, D. A. Carter, *Eur. J. Clin. Microbiol. Infect. Dis.* **2009**, *28*, 1199.
2 E. Mavric, S. Wittmann, G. Barth, T. Henle, *Mol. Nutr. Food Res.* **2008**, *52*, 483; C. J. Adams, M. Manley-Harris, P. C. Molan, *Carbohydr. Res.* **2009**, *344*, 1050.
3 P. C. Molan, *Int. J. Low. Extrem. Wounds* **2006**, *5*, 40; A. Lotfi, *Res. J. Biol. Sci.* **2008**, *3*, 136; B. G. Visavadia, J. Honeysett, M. H. Danford, *Br. J. Oral Maxillofac. Surg.* **2008**, *46*, 55.
4 C. Vuong, M. Otto, *Microbes Infect.* **2002**, *4*, 481.
5 M. D. Blanco, R. Olmo, J. M. Teijon, in *Encyclopedia of Pharmaceutical Technology*, 2nd ed., ed. by J. Swarbrick, Marcel Dekker, New York, **2004**, pp. 239–259.
6 M. E. Rupp, G. L. Archer, *Clin. Infect. Dis.* **1994**, *19*, 231.
7 M. P. Kalapos, *Toxicol. Lett.* **1999**, *110*, 145; P. J. Thornalley, A. Langborg, H. S. Minhas, *Biochem. J.* **1999**, *344*, 109.
8 I. R. Booth, G. P. Ferguson, S. Miller, C. Li, B. Gunasekera, S. Kinghorn, *Biochem. Soc. Trans.* **2003**, *31*, 1406; I. Nemet, L. Varga-Defterdarovic, Z. Turk, *Mol. Nutr. Food Res.* **2006**, *50*, 1105.
9 A. D. Russell, *J. Appl. Microbiol. Symp. Suppl.* **2002**, *92*, 16S.
10 M. Ghosh, D. Talukdar, S. Ghosh, N. Bhattacharyya, M. Ray, S. Ray, *Toxicol. Appl. Pharmacol.* **2006**, *212*, 45.
11 D. Talukdar, B. S. Chaudhuri, M. Ray, S. Ray, *Biochemistry (Moscow)* **2009**, *74*, 1059.
12 L. D. Rhein, *Surfactants in Personal Care Products and Decorative Cosmetics*, CRC/Taylor & Francis, Boca Raton, **2007**, pp. 325–340.

