Stabilised allicin has now been formulated into an active range of products called Allimed™. Powder capsules, liquid, soap and cream presentations all show highly significant activity against multi-drug resistant organisms including MRSA. The mechanism of action of allicin may be due to inhibition of certain thiol-containing enzymes in the microorganisms by the rapid reaction of thiosulfates with thiol groups. This was assumed to be the main mechanism involved in the antibiotic effect of allicin. Recent studies have suggested that the mechanism of action of Allicin may be its ability to react with a model thiol compound (L-cysteine) to form the S-thiolation product S-allylmercapto-cysteine. The identification of the thiolation product was proven by nuclear magnetic resonance as well as by mass spectroscopy. The main antimicrobial effect of allicin is due to its interaction with important thiol-containing enzymes. In the amoeba parasite, allicin was found to strongly inhibit the cysteine proteinases, alcohol dehydrogenases as well as the thioredoxin reductases (Ankri et al., unpublished results) which are critical for maintaining the correct redox state within the parasite. Inhibition of these enzymes was observed at rather low concentrations (<10 mg/mL). Allicin also irreversibly inhibited the well known thiol-protease papain, the NADP+ dependent alcohol dehydrogenase from Thermoanaerobium brockii and the NAD+ dependent alcohol dehydrogenase from horse liver. Figure A shows the effect of allicin on alcohol dehydrogenase activity using 2 different alcohol substrates. This indicates the level of alcohol dehydrogenase activity in the solution. There is a clear relationship between reduction in activity and increasing allicin concentration (mg/l). The concentration of enzyme used in the experiment remained constant.

**Figure 1**: The effect of allicin on alcohol dehydrogenase activity using 2 different alcohol substrates. Absorbance is measured at 340nm.
Inside Story Headline

Alicin is recognised as the main bioactive agent from *Allium sativum* or garlic. This compound is highly active but generally unstable. Using a cold aqueous extraction method, we have obtained a novel extract of allicin (Allimed™) that we have reported is stable and highly active in vitro against methicillin resistant *Staphylococcus aureus* (MRSA). Due to national publicity of Allimed™, patients with long standing unresolved MRSA infections requested this agent for treatment. MRSA is commonly related to delayed closure for many chronic and acute wounds. This is associated with high levels of bacteria in tissues but they can also close through toxin secretion. These toxins can cause local necrosis and disrupt the delicate balance of critical mediators such as cytokines and proteases necessary for healing progression. Allicin has been reported to have a strong SH-modifying and antioxidant properties. Allicin reacts very rapidly with free thiol groups, via a thiol-disulphide exchange reaction. The main antimicrobial effect of allicin may be due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase. In previous work we have demonstrated that allicin is bactericidal against MRSA at concentrations of 128 to 256 ug/ml (Figure 2).

We present initial findings from three patients who have completed a course of treatment. These courses consisted of Allimed™capsules (450mg, 3 per day); spraying liquid Allimed™ (1000 ug ml⁻¹) onto the affected areas once per day and applying Allimed™ Cream (500 ug ml⁻¹) to the infected area once daily.

Patients recruited

Patients were screened, nasal and wound for MRSA prior and during treatment. All patients were nose and wound swab MRSA positive prior to treatment. All were over 60 years of age and had either major surgery or long term skin infections leading to the formation of ulcers infected by MRSA. Two of the MRSA infections were community acquired and one hospital acquired. The strains isolated from each patient were tested in vitro against Allimed™ and all were susceptible. Patients reported an improvement in their condition after 2 and 6 weeks treatment and the infections resolved in 3 to 4 months, see figures 2 and 3.

Although the timescales required for treatment may be longer than those normally required using antibiotics, the initial relief from weeping ulcers and pain was much quicker. It should be noted these the patients had been receiving unsuccessful treatment with antibiotics for months or years prior to treatment with Allimed™.

A possible reason for the initial relief from symptoms could relate to the reported activity of allicin extracts to

Figure 2

Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC/MBC) of Allicin against 30 Clinically Isolated strains of MRSA

<table>
<thead>
<tr>
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<th>MIC 16 mg/l</th>
<th>MIC 32 mg/l</th>
<th>TOTALS</th>
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</thead>
<tbody>
<tr>
<td>MBC 128 mg/l</td>
<td>76%</td>
<td>12%</td>
<td>88%</td>
</tr>
<tr>
<td>MBC 256 mg/l</td>
<td>12%</td>
<td>0</td>
<td>12%</td>
</tr>
<tr>
<td>TOTALS</td>
<td>88%</td>
<td>12%</td>
<td>100%</td>
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No zone was found when using mupirocin cream on at least 30% of MRSA strains tested

Large zones of inhibition are reported with 500ppm allicin as seen in Allimed Cream
Reduced enzyme activity

neutralise bacterial exoenzymes in vitro. In initial studies we have demonstrated that even after brief exposure, allicin can reduce the activity of microbial enzymes. The activity of alcohol dehydrogenase in producing NADH from NAD⁺ was reduced as the concentration of allicin in the solution increased (figure 1). The potential ability to reduce the activity of extracellular virulence factors account for the findings that patients got relief from their symptoms before the MRSA were fully removed from the lesion site.

Chronic leg wound

The initial relief from weeping ulcers and pain was much faster than recorded with pharmaceutical antibiotics

Safety and toxicology confirm allicin is safe

Recent independently conducted safety and toxicological testing of Allicin powder and liquid extracts have confirmed safety of both presentations. Acute oral toxicity studies showed that a single dose of 2000 mg/kg had no adverse effects on Sprague Dawley rats. Tests were conducted in Scottish laboratories. With millions of people already exposed to allicin the safety and side effect profile is generally favourable. Allimed™ can be safely added to almost any other pharmaceutical agent including Warfarin.

All patients are now free from MRSA infection
What else can allicin kill?

A broad range of bacteria, including E. coli, Staphylococcus Aureus, Streptococcus pyogenes, Proteus mirabilis, Pseudomonas aeruginosa, Acetobacter baumanii, Klebsiella pneumoniae, Enterococcus faecium, Myco-bacterium tuberculosis, H. pylori, Salmonella, Clostridium and Shigella are allicin-sensitive. Some of the bacteria listed are killed by allicin concentrations as low as 3-15 ppm (3-15 mcg/ml). However, friendly bacteria such as Lactobacillus, Enterococcus and Pediococcus are concentration dependent to allicin. It has also been noted that allicin is synergistic with antibiotics, and that most bacteria are unable to develop resistance to allicin. Further research also indicates that various multi-drug resistant bacteria are also effectively killed by allicin, some at doses as low as 15-30 ppm (15-30 mcg/ml). Allicin also has a powerful antifungal effect, with a minimum inhibitory concentration (MIC) against various Candida species of only 0.15 to 0.8 mcg/ml. Allicin is effective against other fungal species including Cryptococcus, Trichophyton, Epidermophyton and Microsporum at MIC’s of 1.57-6.25 mcg/ml. Allicin has also shown good anti-viral activity.