Sterilization by gamma radiation of antibiotic impregnated polymethylmethacrylate and plaster of Paris beads

A pilot study

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Keywords
Radiation, sterilization, antibiotics, beads

Summary

Introduction: Ethylene oxide is currently recommended for sterilization of antibiotic impregnated beads; however this method carries health risks to personnel and is becoming less available.

Objective: To perform a pilot study of the effect of radiation for sterilization of polymethylmethacrylate (PMMA) and plaster of Paris (POP) beads impregnated with amikacin, enrofloxacin, and ceftiofur.

Hypothesis: Radiation would effectively sterilize the beads without affecting the efficacy of the antibiotic.

Materials and methods: Beads of PMMA and POP were prepared in a clean but non-sterile manner with one of the three antibiotics (amikacin, enrofloxacin, ceftiofur) or no antibiotic. Beads were then exposed to radiation for a total dose of 0 kiloGray (kGy), 10 kGy and 25 kGy. Beads were incubated on Mueller-Hinton agar plates seeded with Escherichia coli, Staphylococcus aureus or Pseudomonas aeruginosa for 24 hours or cultured in brain-heart infusion broth for 48 hours. Zones of inhibition were measured on the agar plates and statistics were performed on the diameters of the zones of inhibition using an analysis of variance.

Results: There were no differences in the diameters of inhibition for all levels of radiation for all PMMA beads. The same was true with POP beads with the exception of enrofloxacin which had a significantly decreased zone of inhibition with increased levels of radiation, though the clinical significance of this finding was not assessed. Only beads without antibiotics and not exposed to radiation had bacterial growth.

Clinical significance: Radiation may be an effective method of sterilization for antibiotic impregnated beads.

Introduction

Antibiotic impregnated beads of either polymethylmethacrylate (PMMA) or plaster of Paris (POP) are an established method of prevention or treatment of orthopaedic infections in equine surgery. A variety of different antibiotics have been studied for their efficacy and duration of elution, however, only two of these studies included an assessment of sterilization (1–5). Ramos et al. found that ethylene oxide gas sterilization had no effect on metronidazole and gentamicin bioactivity (1). Báez et al. assessed ethylene oxide gas and autoclave sterilization of meropenem impregnated PMMA beads and found that meropenem was inactivated by autoclaving (5). Many commonly used antibiotics have not been studied so the effect of high temperatures is not established. The concern over susceptibility to high temperatures has led to the general recommendation in most textbooks of equine surgery to sterilize antibiotic impregnated beads by ethylene oxide gas (6). However, recent large epidemiological studies have revealed potential health hazards associated with personnel who work closely with and are exposed to ethylene oxide gas sterilization (7, 8). These risks include an observed excess bone cancer mortality (based on small numbers) and positive exposure-response trends for breast cancer in women and lymphoid cancer in men (7, 8). Based on these studies, some countries and institutions have begun to phase out or limit sterilization by ethylene oxide gas. In our insti-
Radiation sterilization of antibiotic-impregnated beads

Studying the effect of radiation on microstructures (17). No studies could be found regarding the effect of radiation on POP or PMMA beads although it has been studied regarding the effect of radiation on POP or PMMA beads impregnated with antibiotics that require study. The objective of this study was to perform a small pilot project on the use of gamma radiation for sterilization of PMMA or POP beads impregnated with antibiotics. The hypothesis was that radiation will be effective at sterilizing the beads without affecting the action of the antibiotics.

Materials and methods

Beads of PMMA and POP were prepared with either no antibiotics, ceftiofur, enrofloxacin, or amikacin. Beads were not prepared under sterile conditions. Non-sterile gloves were worn during preparation. For each 20 grams of PMMA powder, one of the following was added:

- 1 gram of amikacin (with 8 ml sterile water for total 12 ml),
- 1 gram of enrofloxacin (10 ml)
- 1 gram of ceftiofur in 12 ml of sterile water
- 10 ml sterile water

For each 20 grams of PMMA powder, one of the following was added:

- 1 gram of amikacin (in 12 ml)
- 1 gram of enrofloxacin (10 ml)
- 1 gram of ceftiofur in 12 ml of sterile water
- no antibiotics (just liquid monomer)

Eighty beads of each combination were made. Each combination was mixed for two minutes. To ensure uniformity, the POP was placed into a preformed mould.

To assess sterility, beads from all groups were placed into 5 ml of Brain Heart infusion Broth and incubated at 35 degrees Celsius for 48 hours to check for bacterial growth. For evaluation of antibacterial activity, all combinations of antibiotic impregnated beads with or without sterilization by radiation were evaluated by disk diffusion test. Briefly the beads were placed onto Mueller-Hinton agar plates previously inoculated with 0.5 McFarland turbidity standard (106 CFU/ml) of Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 or Pseudomonas aeruginosa ATCC 27853. The diameters of the zones of inhibition were then measured and recorded in a spreadsheet program after 24 hours of incubation at 35°C.

Statistical analysis was performed on the diameters of inhibition for each antibiotic and radiation level by using an analysis of variance (ANOVA) with the level of significance set at p <0.05. The factor to be analyzed was the effect of radi-

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When plated with *S. aureus* with an increasing level of radiation (*p* = 0.0009) (▶Table 1). The difference was significant between all levels of radiation (▶Table 2).

All beads had very low variability between the diameters of the zones of inhibition with the exception of PMMA impregnated with *ceftiofur*, which appeared to have an increased variability when plated against *P. aeruginosa*. This variability was not affected by the level of radiation (▶Table 3).

**Discussion**

This preliminary study was intended to investigate the potential use of sterilization by radiation for antibiotic impregnated heart infusion. No bead that was exposed to either 10 or 25 kGy of radiation had bacterial growth including those that did not contain antibiotics. Internal dosimetry controls confirmed in all cases that the beads received the accurate dose of radiation.

There was no significant difference between the diameters of the zones of inhibition with the exception of PMMA impregnated with *ceftiofur*, which appeared to have an increased variability when plated against *P. aeruginosa*. This variability was not affected by the level of radiation (▶Table 3).

### Results

All beads that underwent sterilization with radiation were subjectively judged to have an increasing amount of brownish discolouration with an increasing level of radiation. Only those beads that contained no antibiotic and were not sterilized had bacterial growth (*Bacillus cereus*) in the brain-heart infusion. No bead that was exposed to either 10 or 25 kGy of radiation had bacterial growth including those that did not contain antibiotics. Internal dosimetry controls confirmed in all cases that the beads received the accurate dose of radiation. Before statistical analysis was performed the data were tested for normal distribution using an Anderson Darling test and equality of variance using a Levene's test. A post-hoc students t-test was performed on significant outcomes from the ANOVA to determine the specific interaction between each level of radiation. The alpha level for each comparison was adjusted using a sequential correction.

### Table 1 Results of the analysis of variance (with the variable of the average diameter of the zone of inhibition for each individual group of bead type and antimicrobial plated against a bacteria compared to the variable level of radiation). * Indicates a significant result.

<table>
<thead>
<tr>
<th>Bead type</th>
<th>Antimicrobial</th>
<th><em>Escherichia coli</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymethylmethacrylate</td>
<td>Amikacin</td>
<td><em>p</em> = 0.187</td>
<td><em>p</em> = 0.092</td>
<td><em>p</em> = 0.054</td>
</tr>
<tr>
<td></td>
<td>Ceftiofur</td>
<td><em>p</em> = 0.533</td>
<td><em>p</em> = 0.550</td>
<td><em>p</em> = 0.115</td>
</tr>
<tr>
<td></td>
<td>Enrofloxacin</td>
<td><em>p</em> = 0.774</td>
<td><em>p</em> = 0.382</td>
<td><em>p</em> = 0.352</td>
</tr>
<tr>
<td>Plaster of Paris</td>
<td>Amikacin</td>
<td><em>p</em> = 0.308</td>
<td><em>p</em> = 0.085</td>
<td><em>p</em> = 0.868</td>
</tr>
<tr>
<td></td>
<td>Ceftiofur</td>
<td><em>p</em> = 0.056</td>
<td><em>p</em> = 0.072</td>
<td><em>p</em> = 0.175</td>
</tr>
<tr>
<td></td>
<td>Enrofloxacin</td>
<td><em>p</em> = 0.290</td>
<td><em>p</em> = 0.0009*</td>
<td><em>p</em> = 0.097</td>
</tr>
</tbody>
</table>
Table 2  The zones of inhibition shown with ± standard deviation in mm for plaster of Paris beads impregnated with amikacin, enrofloxacin and ceftiofur when incubated on Mueller-Hinton plates seeded with *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and increasing levels of radiation from 0 to 25 kiloGray (kGy). There was a significant difference with increasing levels of radiation for enrofloxacin beads plated with *Staphylococcus aureus*. From 0–10 kGy, p = 0.026 ; from 10–25 kGy, p = 0.04 ; and from 0–25 kGy, p = 0.0016. A significant difference between each is indicated by the different superscript letters.

<table>
<thead>
<tr>
<th>Plaster of Paris</th>
<th><em>Escherichia coli</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>38 ± 1.07</td>
<td>41 ± 0.71</td>
<td>45 ± 1.30</td>
</tr>
<tr>
<td>10 kGy</td>
<td>39 ± 0.74</td>
<td>42 ± 0.71</td>
<td>45 ± 0.71</td>
</tr>
<tr>
<td>25 kGy</td>
<td>38 ± 0.64</td>
<td>41 ± 0.35</td>
<td>45 ± 0.64</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>45 ± 1.49</td>
<td>45 ± 1.49a</td>
<td>39 ± 0.64</td>
</tr>
<tr>
<td>10 kGy</td>
<td>44 ± 0.71</td>
<td>43.5 ± 1.31b</td>
<td>39 ± 0.93</td>
</tr>
<tr>
<td>25 kGy</td>
<td>44 ± 1.38</td>
<td>41 ± 2.26c</td>
<td>40 ± 0.88</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>42.5 ± 0.53</td>
<td>48 ± 1.13</td>
<td>38 ± 1.45</td>
</tr>
<tr>
<td>10 kGy</td>
<td>42.5 ± 0.53</td>
<td>49 ± 1.00</td>
<td>37 ± 0.64</td>
</tr>
<tr>
<td>25 kGy</td>
<td>42 ± 0.88</td>
<td>49.5 ± 1.10</td>
<td>37 ± 0.64</td>
</tr>
</tbody>
</table>

PMMA or POP beads after the use of ethylene oxide sterilization was discontinued at our institution. The study successfully established that the antibiotic impregnated POP or PMMA beads did retard bacterial growth of three different bacteria after sterilization by radiation. This would serve as an indication that the efficacy of the antibiotics was unaffected (with the exception of enrofloxacin in POP) by the radiation. Additionally, the radiation appeared to effectively sterilize the beads as only unsterilized beads had bacterial growth after preparation, despite all beads being prepared in a non-sterile manner.

The variation in zones of inhibition of *P. aeruginosa* from ceftiofur incorporated into PMMA is difficult to explain with the current study. A technical error in the mixing of the beads does not explain the variability as the variability was not present regardless of radiation level. There may also be an inherent difference that has been studied after radiation with out showing the same effect, but this change requires further investigation looking for products of radiolysis (11). Further studies looking at the concentrations of enrofloxacin release from POP or PMMA beads as well as HPLC to look for products of radiolysis is indicated.

Table 3  The zones of inhibition shown with ± standard deviation in mm for polymethylmethacrylate beads impregnated with amikacin, enrofloxacin and ceftiofur when incubated on Mueller-Hinton plates seeded with *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and increasing levels of radiation from 0 to 25 kiloGray (kGy). There was an increased amount of variability in the ceftiofur impregnated beads when plated against *Pseudomonas aeruginosa* regardless of radiation level.

<table>
<thead>
<tr>
<th>Polymethylmethacrylate</th>
<th><em>Escherichia coli</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>17 ± 1.13</td>
<td>20 ± 1.51</td>
<td>19 ± 0.83</td>
</tr>
<tr>
<td>10 kGy</td>
<td>18 ± 0.93</td>
<td>21 ± 1.49</td>
<td>20 ± 0.88</td>
</tr>
<tr>
<td>25 kGy</td>
<td>17 ± 0.74</td>
<td>22 ± 1.67</td>
<td>20 ± 0.74</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>39 ± 0.71</td>
<td>35 ± 0.99</td>
<td>30 ± 0.52</td>
</tr>
<tr>
<td>10 kGy</td>
<td>39 ± 0.76</td>
<td>35 ± 1.04</td>
<td>31 ± 0.99</td>
</tr>
<tr>
<td>25 kGy</td>
<td>39 ± 0.93</td>
<td>35 ± 0.74</td>
<td>31 ± 1.07</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>29 ± 1.36</td>
<td>32 ± 0.89</td>
<td>14 ± 3.54</td>
</tr>
<tr>
<td>10 kGy</td>
<td>28.5 ± 0.93</td>
<td>31 ± 1.39</td>
<td>18 ± 3.48</td>
</tr>
</tbody>
</table>
| 25 kGy                  | 29 ± 1.46           | 32 ± 1.25               | 17 ± 3.89                 

There was a significant decrease in efficacy of enrofloxacin in POP beads against *S. aureus* with increasing levels of radiation. This may be considered a sign that enrofloxacin is sensitive to degradation by increasing radiation. The same effect was not seen with PMMA beads. Again, a potential increased release of enrofloxacin from the POP beads in the first 24 hours of incubation may have revealed a radiosensitivity that was not evident when smaller quantities were released from PMMA beads. There may also be an inherent difference in the exposure to radiation of the enrofloxacin in POP versus PMMA. There are other matrices containing enrofloxacin that have been studied after radiation without showing the same effect, but this change requires further investigation looking for products of radiolysis (11). Further studies looking at the concentrations of enrofloxacin release from POP or PMMA beads as well as HPLC to look for products of radiolysis is indicated.

The study did possess several limitations as it was designed as a pilot study to investigate the possibility of using radiation to sterilize antibiotic impregnated beads due to a lack of access to either ethylene oxide or HPGP sterilization at our institution. Radiation is known to cause the production of free radicals and products of bacterial contamination of the plates. The radiation level also does not appear to play a role as the variation was constant across radiation levels and the radiation was delivered in a consistent manner as verified by internal controls. The use of high performance liquid chromatography (HPLC) to determine the concentration of ceftiofur release may provide an explanation if the release of the ceftiofur is inconsistent from PMMA or if the efficacy against *P. aeruginosa* is limited and variable. The same variation was not seen with POP beads impregnated with ceftiofur. It appears that the release of other antibiotics from POP is generally faster than from PMMA, thus the POP-ceftiofur beads may have had a higher concentration of ceftiofur released during the first 24 hours of incubation and were more efficacious while a smaller concentration released from the PMMA beads may have been less efficacious and variable against the bacteria (2, 3).
radiolysis. The presence or absence of free radicals was not investigated by the use of electron spin resonance and would be important to determine in the future before clinical use. The use of HPLC would also have enabled further investigation of the antibiotic release from the beads over time and if there were degradation products present from interaction between the radiation and the antibiotic. The water content was not assessed in this study, and, as water is a source of free radicals, this could be indirectly assessed by the production of free radicals, or by direct measurement of water content in future studies. As the reproducibility of radiation sterilization will be influenced by the water content of the materials being sterilized, future studies should include beads with known and constant water content.

The dose of 25 kGy is the accepted standard level of radiation required for most sterilization procedures and is the recommended dose (18). However the level of radiation truly required for sterilization needs investigation for each substance and depends on the biocontamination before sterilization, the radiosensitivity of the organisms, and the sterility assurance level. The sterility assurance level is the probability that an organism survives after exposure to the validated sterilization procedure. The sterilization of POP and PMMA beads therefore also requires investigation using a pre-sterilization inoculum of bacteria and increasing levels of radiation. The lowest level of radiation that would kill the contaminating bacteria while minimising the damage would be ideal for that product. This level has yet to be established with POP and PMMA.

The brownish discolouration of the beads has previously been reported after sterilization by radiation and has not been noted to have an association with changes in efficacy. This was only subjectively analyzed in this study as it is an established side effect of radiation (16).

The alternative of hydrogen peroxide gas plasma sterilization also justifies investigation to determine the level of hydrogen peroxide present after sterilization and if that would have a beneficial or negative effect on the tissues after implantation. This method was not available to our institution and was not included in the study. Therefore this pilot study established that radiation is a potential option for sterilization of antibiotic impregnated PMMA or POP beads. Amikacin, enrofloxacin and cefotiofur included in the beads remained effective at the single time point studied after radiation although enrofloxacin appeared radiosensitive when combined with POP. Further investigation needs to be performed to investigate potential negative effects of that radiation.

Acknowledgements

Our thanks to Bruno Laventure and the Centre de Recherche of Agriculture and Agroalimentaire Canada for the sterilization by radiation. This project was supported by the Fonds de Recherche Clinique de Pfizer Santé Animale.

Conflict of interest

None to declare.

References


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