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Catheter related bacteremia in rats: A preliminary report on the effect of methylene blue coated catheter

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The safety and efficacy of methylene blue (MB) coated indwelling jugular vein/cranial vena cava catheter made up of polyurethane material was tested in a rat model, receiving bacterial culture suspension of Pseudomonas aeruginosa, and Staphylococcus aureus. Daily blood samples were collected from the catheter and peripheral vein for bacterial culture. The clinical parameters (rectal temperature, respiratory rate, total white blood cell count, and loss in body weight) were not different between the groups. All the rats became bacteremic with similar changes in the number of colony forming units in the catheter and peripheral samples. Histopathological lesions were not different between the groups. The findings suggest that rats receiving MB coated catheters behaved similar to non-coated catheters. Based on the results it can be concluded that for this type of gross contamination, catheter coating alone may not eliminate infection/bacteremia.

Keywords: Catheter, Jugular Vein, Methylene Blue, Rat

The antibacterial properties (preventing bacterial colonization) of methylene blue (MB), a phenothiazine dye and a potent inhibitor of guanylate cyclase, are recognized. However, its effects on controlling the infection by the common organisms that are grown in central venous catheters are not known. Prior to its application in controlling catheter related blood stream infections in humans, the efficacy of MB in controlling gross bacterial contamination in an in vitro model has to be tested. The aims of the present study have been three-fold: (a) to determine whether there is a bactericidal effect of MB impregnation of polyurethane on bacteria in proximity, (b) to test whether an MB coated polyurethane catheter that is placed in a rat’s jugular vein/cranial vena cava can reduce the multiplication of Staphylococcus aureus and Pseudomonas aeruginosa after intraluminal contamination, and most importantly (c) to determine whether it is safe to use an MB coated catheter and to determine adverse effects, if any, to the surrounding jugular vein/cranial vena cava and tissue, and other vital clinical parameters. Since Staphylococcus aureus is an important cause of central venous catheter-associated bacteremia, this organism and Pseudomonas aeruginosa were utilized for this study. A rat model described earlier was chosen for its quantitative outcome in a short time for a study of this nature. Further, the intravascular catheter-associated infection in this rat model appears to mimic the human condition.

Materials and Methods

In vitro studies

In the first experiment, polyurethane plastic (EG-85A from Tecoflex family of aliphatic polyurethane), impregnated with MB by employing ascorbic acid as an activating agent was tested for its efficacy to control bacterial growth. Following three strains of bacteria obtained from ATCC were used: Escherichia coli 25922, Staphylococcus epidermidis 49134, and Staphylococcus aureus 29213. The culture of the selected strain was placed on the bottom of one plate of the Petri dish and covered by a piece (2.25 cm × 2.25 cm) of coated material. The Petri dish was inverted and a small container with 1 ml of water was placed on the bottom of the other plate of the Petri dish prior to incubation to prevent drying of the culture medium. The Petri dish was placed in the upside position in an incubator for 24 hr at 37°C. Two controls were cre-
ated, one was a piece (2.25 cm × 2.25 cm) of polyurethane without any MB coating and the second one was a 75 µl bacteria culture placed in a Petri dish as a ‘hanging drop’. After 24 hr of incubation at 37°C, 15 µl bacterial culture from each plate was mixed with 15 ml of fresh trypticase soy medium and serially diluted prior to overnight incubation at 37°C to evaluate colony forming units (CFU). In the second experiment, pieces (1.5 cm × 1.5 cm) of polyurethane impregnated materials were placed in 20 ml of normal saline at room temperature and the release rate of MB into saline was determined for 4 months. The amount of MB in mg that released was determined every other day during the first 20 days, and at 40, 80 and 120 days.

In vivo study

Animals—The animal experiments were performed in adherence to the National Institutes of Health guidelines on the use of experimental animals. Approval was obtained from the Purdue University Animal Care and Use Committee prior to the initiation of the experiment. Pathogen-free male Spraque-Dawley rats (Charles River, Wilmington, MA) with an average body weight of 430 g were used.

Catheters—Polyurethane tubing (outer diameter 1 mm and inner diameter 0.6 mm) of approximately 20 cm long was impregnated with MB employing ascorbic acid as an activating agent. A ‘retention bead’ of 3 mm size was created outside the tubing approximately 4 cm from the tip by adding a clear sealant. This enabled to fix the catheter to the jugular vein.

Surgery—Rats (24) underwent jugular vein catheterization as described elsewhere with certain modifications. After induction of anaesthesia and surgical preparation, the right jugular vein was exposed. Venotomy was made approximately 5 mm cranial to the site of crossover by the pectoralis major muscle. The catheter filled with heparinized dextrose was inserted into the jugular vein and advanced to reach the cranial vena cava such that the retention bead sat on the venotomy site. The approximate distal length of the catheter from the retention bead was 4 cm. The retention bead was secured to the jugular vein by silk sutures. The distal portion of the catheter was tunneled subcutaneously to exit on the mid-line of the dorsal surface 4 cm anterior to the ears. Approximately 4 cm of the catheter was left outside the body of the rat and the tip was clamped using a small stainless steel he- moclip (WECK Hemoclip Traditional Legating Clips, WECK Closure Systems, Research Triangle Park, NC 27709). The skin incision was closed by 5-0 absorbable vicryl coated suture material. Normal saline (2 ml) was given subcutaneously to the rats immediately after surgery. Twelve rats (control group-CONT) received a non-coated catheter, and 12 other rats (treatment group-TRT) received a catheter that was coated with MB. Day of the surgery was designated as ‘day 0’. The total volume of fluid in the catheter after trimming the excess was approximately 35 µl.

Bacterial culture—Inoculums of specific strains of Pseudomonas aeruginosa and Staphylococcus aureus were prepared overnight from a single colony. Six rats each from CONT and TRT groups received a bacterial culture suspension (in 70 µl sterile saline) of Pseudomonas aeruginosa (10⁶ CFUs/ml). Similarly six other CONT and six of the TRT rats received a bacterial culture of Staphylococcus aureus (10⁶ CFUs/ml) on day 1 of the study. The bacterial suspension was allowed to dwell within the catheter for 30 min before the catheter was flushed with 100 µl of lock solution.

Blood samples and data collection—On day 1 (prior to the injection of bacterial culture into the catheter) and on days 2, 3, and 4, daily samples were collected from the jugular catheter and from the peripheral vein for bacterial culture work. Each time a sample (100 µl) was withdrawn from the catheter followed by injection of 100 µl of lock solution. A peripheral blood sample (100 µl) was collected from the saphenous vein by an established method. Each day body (rectal) temperature and respiratory rates were recorded and the condition of the rats was monitored. On day 4, the rats were weighed before they were euthanized by anaesthetic overdose.

Samples of blood collected for bacterial culture work were immediately diluted 10 times with sterile saline to prevent coagulation. The samples were further serially diluted and each dilution (0.5 ml) was smeared on trypticase soybean agar plates, incubated for 24 hr at 37°C. Colonies were counted and results multiplied by appropriate dilution.

Necropsy and histopathologic examination—On day 4, after the collection of blood samples, the rats were euthanized and necropsy was performed. The tissues surrounding the catheter and vessels were carefully dissected free and were fixed by immersion in 10% neutral buffered formalin. Following fixation, the catheter was removed by gentle traction and transverse sections of jugular and/or anterior vena
cava and surrounding tissues were processed by routine methods, stained with hematoxylin and eosin, and examined microscopically. A pathologist (GWS), blinded to catheter identity, evaluated all tissue sections. Lesions were graded for severity and extent of vascular degeneration and necrosis, presence and relative number of bacteria, presence or absence of thrombosis as well as extent and severity of perivascular cellular inflammation. Points were assigned for each of the following features and the sum formed a score. Mild or focal mural necrosis 1 and focal or diffuse transmural necrosis 2; few bacteria 1 and many (dense colonies of) bacteria 2; thrombus 2, mild perivascular inflammation 1 and marked perivascular inflammation 2. Normal anatomy was graded as zero. Minimum score was 0 and maximum was 8.

*Analysis of data*—The CFUs/ml were log₁₀ normalized prior to the analysis of data. The probability of significant differences in the number of colony forming units for the two types of organisms between the CONT and TRT groups in catheter and peripheral blood samples for days 0, 1, 2, 3, and 4 were analyzed separately by an analysis of variance for repeated measures. Similarly, the changes in the body temperature and respiratory rate for days 1, 2, 3, and 4 between the CONT and TRT groups were analyzed. The computation utilized the statistical analysis system explained in the SAS User’s Guide for analysis of data involving repeated measures. An unpaired *t* test was used to detect the differences in the body weight on day 0 and day 4 between the CONT and TRT groups. A similar test was applied to detect the differences in the total number of white blood cells counted and the necropsy results. Probability values of alpha less than or equal to 0.05 were considered to be significant for these analyses. Data are presented as mean ± SE.

**Results**

*In vitro studies*

All three strains of bacteria exhibited a remarkable inhibition in growth after contacting MB-coated polyurethane plastic. The log₁₀ CFU reduction after 24 hr of incubation was in the range of 7 to 6. The controls had 10⁷ to 10⁸ bacteria per ml. In the second experiment a significant amount (0.1-0.5 mg) of MB released in the first three weeks.

*In vivo study*

**Clinical parameters**—On an average, the rats lost 30 g of body weight during the study. The body temperature (°C) in the CONT group rats on day 1 was 36.69 ± 0.29 and on day 4 was 36.67 ± 0.14. In the TRT group rats the body temperature on day 1 was 35.97 ± 0.23 and on day 4 was 36.59 ± 0.13. The respiratory rate in the CONT group rats on day 1 was 115.5 ± 1.23 and on day 4 was 127.6 ± 0.85. The respiratory rate in the TRT group rats on day 1 was 122.4 ± 1.11 and on day 4 was 128.9 ± 0.92. Body weight (g) in the CONT group rats on day 0 was 429.7 ± 11.00 and on day 4 was 395.4 ± 9.84. In the TRT group rats the body weight on day 0 was 425.3 ± 10.21 and on day 4 was 397.6 ± 6.23. The total number of white blood cells counted (10³/μl) in the CONT group rats on day 0 was 6.21 ± 0.27 and on day 4 was 8.22 ± 1.26. In the TRT group rats the total number of white blood cells counted on day 0 was 7.57 ± 0.59 and on day 4 was 9.85 ± 1.64. All these parameters were not different between the groups. However, the loss in the body weight from day 1 to day 4 was significant (*P* < 0.05) within each group. Barring one catheter, the rest of the catheters were patent over the duration of study. In a few cases additional flushing with lock solution was necessary to regain patency and allow blood to be drawn from the catheter. One rat was excluded from the study because of irreversible clotting of the catheter. None of the rats developed any signs of sepsis despite all becoming bacteremic.

**Bacterial culture results**—The summary on the number of CFUs counted in catheter and peripheral samples for the two organisms in CONT and TRT groups are provided in Fig. 1. As can be seen from the figure, the rats quickly became bacteremic with moderate levels of bacteria in peripheral blood (10³ - 10⁴ CFUs/ml). Changes and trends in CFUs were similar between both groups. The CFUs from catheter samples in both groups started to increase (*P* > 0.05) on day 2, reached their peak on day 3, and maintained this increase on day 4. Increases in the peripheral samples were not different across days. However, the CFUs in catheter samples were higher (*P* < 0.05) compared to the peripheral samples in both groups. In general, the MB coated catheters showed a small but not significant decrease in the growth of *Staphylococcus aureus* by about one log versus the control group.

| Table 1—The combined pathology scores for the different groups |
|-------------|-------------------|
| Group      | Combined path score |
| CONT-S     | 5.6               |

In the TRT group rats the body temperature on day 1 was 36.69 ± 0.29 and on day 4 was 36.67 ± 0.14. In the TRT group rats the body temperature on day 1 was 35.97 ± 0.23 and on day 4 was 36.59 ± 0.13. The respiratory rate in the CONT group rats on day 1 was 115.5 ± 1.23 and on day 4 was 127.6 ± 0.85. The respiratory rate in the TRT group rats on day 1 was 122.4 ± 1.11 and on day 4 was 128.9 ± 0.92. Body weight (g) in the CONT group rats on day 0 was 429.7 ± 11.00 and on day 4 was 395.4 ± 9.84. In the TRT group rats the body weight on day 0 was 425.3 ± 10.21 and on day 4 was 397.6 ± 6.23. The total number of white blood cells counted (10³/μl) in the CONT group rats on day 0 was 6.21 ± 0.27 and on day 4 was 8.22 ± 1.26. In the TRT group rats the total number of white blood cells counted on day 0 was 7.57 ± 0.59 and on day 4 was 9.85 ± 1.64. All these parameters were not different between the groups. However, the loss in the body weight from day 1 to day 4 was significant (*P* < 0.05) within each group. Barring one catheter, the rest of the catheters were patent over the duration of study. In a few cases additional flushing with lock solution was necessary to regain patency and allow blood to be drawn from the catheter. One rat was excluded from the study because of irreversible clotting of the catheter. None of the rats developed any signs of sepsis despite all becoming bacteremic.
Histopathological examination — Average lesion score for rats in the non-coated- *Staphylococcus aureus* (CONT-S) group was 5.6 (with a range of 4-8), for MB-coated- *Staphylococcus aureus* (TRT-S) was 6.2 (with a range of 4-8), for non-coated- *Pseudomonas aeruginosa* (CONT-P) was 4 (with a range of 3-6), and for MB-coated-*Pseudomonas aeruginosa* (TRT-P) was 3.6 (with a range of 2-5). Lesions were relatively severe in CONT-S and TRT-S rats compared to CONT-P and TRT-P rats (Table 1). Lesions were less severe in rats that received *Pseudomonas aeruginosa*. Average lesion score was 5.9 for rats that received *Staphylococcus aureus* and 3.8 for rats that received *Pseudomonas aeruginosa*.

Severe lesions in the TRT-S and CONT-S rats were characterized by discontinuous or absence of endothelium, multifocal or diffuse transmural necrosis, intramural and perivascular infiltrates of viable and degenerate neutrophils, macrophages, lymphocytes and plasma cells as well as perivascular hemorrhage and fibrosis. In certain cases, severe necrotizing phlebitis was noted along with other lesions (Fig. 2a, b). Less severe lesions (mild phlebitis) in the TRT-P and CONT-P rats were characterized by similar, but less severe and less frequent changes (Fig. 2c, d).

There was no difference between CONT-S and TRT-S rats, and between CONT-P and TRT-P rats (*P* > 0.05).

Discussion

The concept of coating catheters with a bacteriostatic or bactericidal substance is not new but the use of MB for this purpose is a novel approach to the problem of central venous catheter colonization and infection. Negligible changes both in CONT and TRT groups in the rectal temperature and respiratory rates over the study period suggest that the MB coated catheter was safe to use in rats. The initial total white blood cell count agreed with Singh et al. Similar changes in the white blood cell count between the groups coupled with lack of clinical differences lend credence to the safe use of the MB coated catheter.

The number of CFUs between the groups was not different for both the organisms (*Pseudomonas aeruginosa, Staphylococcus aureus*) that were studied,
though there was a trend towards lower bacterial concentration in the catheters with the *Staphylococcus aureus* group. There was a persistently higher CFUs from catheter versus peripheral, confirming that catheter remained the source of bacteremia. It was not expected to have complete elimination of colony forming units from both catheter and peripheral samples in TRT rats or the CONT rats due to large inoculum of bacteria to the catheter lumen, but the number was expected to decrease significantly, based on *in vitro* studies. The reason for lack of effectiveness of the MB coating to reduce bacterial contamination in this study is not known. Factors such as presence or absence of blood and periodic sample withdrawal may have contributed to this muted response in the *in vivo* study compare to the *in vitro* study. However, a higher concentration of MB may have restored antibacterial effectiveness. In addition, to offset the effects of blood contact within the catheter, use of an antibacterial lock solution containing high concentration of MB (in citrate) will be necessary. Although *in vitro* work suggested that catheters can deliver the MB into surrounding saline for a prolonged period of time delivery into biofilm layers is unknown. Biofilm is formed around the bacteria on the surface of the catheter over a period of time. The formation of biofilm appears to be a two step process that requires adhesion of the bacteria to the bacterial surface fol-
allowed by cell-to-cell accumulation and biofilm formation\textsuperscript{11}.

Histopathological evaluation of the jugular vein/cranial vena cava and surrounding tissue is essential to determine the pathologic damage, if any, due to the presence of an MB coated catheter within the jugular vein/cranial vena cava. The evaluation can be done in different ways. We adopted a system of scoring that gave points to different lesions as opposed to a scoring system that is based on the severity of the lesion\textsuperscript{10}. The lesions were similar between CONT and TRT rats, with more severe lesions in rats that received \textit{Staphylococcus aureus} than those receiving \textit{Pseudomonas}. Overall, the lesions observed in all the rats were quite severe for a study that lasted only for 4 days.

In conclusion, this study demonstrated the safe use of an MB treated catheter for a short period of time in a rat model of catheter related bacteremia. The inability to control the bacterial growth by the MB catheter despite the strong evidence that was demonstrated in the \textit{in vitro} study is not known. Many hypotheses for future studies can be developed based on this different outcome between \textit{in vitro} and \textit{in vivo} studies. These hypotheses will be tested in addition to experiments that will determine the effectiveness of an antibacterial lock solution of MB/citrate.

References