Effects of high antibiotic concentrations on human umbilical vein endothelial cells

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INTRODUCTION:

Continuous local antibiotic perfusion (CLAP) has recently attracted attention as a novel drug delivery system for orthopaedic infections [1-3]. CLAP includes direct continuous infusion of high-concentration gentamicin (GM) into the bone marrow and soft tissue, however its influence on vascular endothelial cells (HUVECs) has not been well studied. In this study, we examined the effects of high antibiotic concentrations on HUVECs.

METHODS:

***Cell preparation*:** HUVECs were purchased from Kurabo (Osaka, Japan) and cultured in an endothelial cell growth medium. Cells were maintained in a humidified incubator at 37℃ with 5% CO_2, with medium changed every 3-4 days.

***Cell viability assay*:** A total of 3×10^3 HUVECs per well were seeded in a 96-well plate and incubated for 24 h and then exposed to GM at 12 concentrations ranging from 0 to 10,000 μg/mL. Cell viability was assessed on days 4, 7, and 14 using the Cell Counting Kit-8 from Dojindo Lab (Kumamoto, Japan) ; values were normalized to the pre-exposure baseline (100%). In addition, restoration of cell viability was checked after GM exposure. Cells cultured with GM for 14 days were washed and further incubated for 14 days in GM-free medium, the absorbance values at the end of the GM exposure and at the end of the total culture were evaluated.

***Apoptosis assay*:** A total of 1×10^4 HUVECs per well were seeded into 6-well plates and incubated in a growth medium for 24 h. The cells were then exposed to GM at 9 concentrations ranging from 0 to 10,000 μg/mL for 48 h, and performed apoptosis assay using Apo-direct TUNEL Assay Kit (Phoenix flow systems, Arizona). The apoptosis rate was semi-quantified as the ratio of terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) stained nuclear fragmentation to 4′,6-diamidino-2- phenylindole (DAPI) stained cells.

***Tube formation assay*:** HUVECs were exposed to GM at 6 concentrations ranging from 0 to 10,000μg/mL for 4, 7, and 14 days. After incubating 100 μL of matrigel spread on a 48-well plate for 3 h, exposed HUVECs were reseeded onto the gel at a density of 8.0×10^4 per well. After 15 h of incubation, images were captured using an electron microscope to evaluate vascular formation ability. The average branching length and number of junctions in the captured images were analyzed using ImageJ.

RESULTS:

***Cell viability assay*:** Exposure to GM concentrations of 3,000 μg/mL or more significantly decreased cell viability between days 4 and 14 (Fig. 1a). Every timepoints could see the trend that the cell viability decreases as GM concentration increase. Cell viability was significantly decreased by GM concentration of 2,000 or more on day 14 (Fig. 1b). For the restoration of cell viability, cells cultured in normal medium for 14 days after 14 days of GM exposure showed a significant increase at GM concentrations below 1,000 μg/mL compared with cells immediately after 14 days of GM exposure. (Fig. 1c).

***Apoptosis assay*:** Apoptosis assay showed significant higher apoptosis rate at GM concentrations above 2,000 μg/mL (Fig 2).

***Tube formation assay*:** In the tube formation assay, a decrease of vascular formation ability was observed as the exposure day to GM increased. In the 14-day group, both the average branching length and number of junctions decreased significantly at concentrations above 500 μg/mL (Fig 3a, b).

DISCUSSION:

In this study, GM had a negative effect on cell proliferation, restoration, and apoptosis rates at concentrations above 2000 μg/mL, and on angiogenesis at concentrations above 500 μg/mL.

CLAP therapy in clinical practice generally involves continuous administration of 1,200 μg/mL GM, and the average duration of CLAP therapy is approximately 14 days [1]. According to our previous report focusing the effect of GM concentration for human bone tissue-derived cells, increased GM concentration interfered osteogenic potential and mineralization potential [4]. They reported GM concentration of 250 μg/mL or more significantly decreased the cell viability on day 14. Then, there is possibility that vascular endothelial cells have more resistance than osteogenic cells.

The results of the tube formation assay indicate angiogenesis of the cells were affected by lower concentrations than proliferations, therefore it is supposed that the function of HUVECs may be negatively affected at lower GM concentrations compared to their proliferative potential. We should further investigate the effect for the function by genetic assay and migration assay not only tube formation assay.

SIGNIFICANCE: The current study demonstrates that high concentrations of GM negatively affect cell function. Further studies are required to determine the optimal antibiotic concentration for CLAP therapy.

REFERENCES: [1] Maruo A, et al. J Orthop Surg. 2021. [2] Oe K, et al. J Orthop Case Rep. 2021. [3] Maruo A, et al. J Orthop Surg. 2022. [4] Yamamoto Y, et al. Bone Joint Res. 2024

Fig. 1a

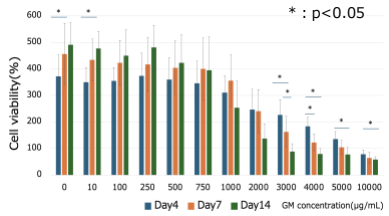

Fig. 1b

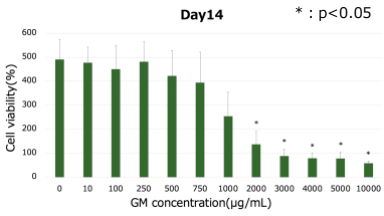

Fig. 1c

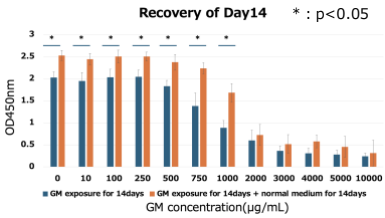

Fig. 2

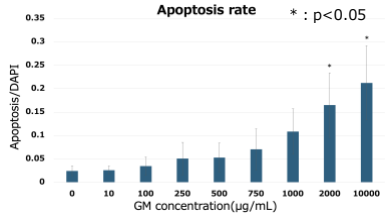

Fig. 3a

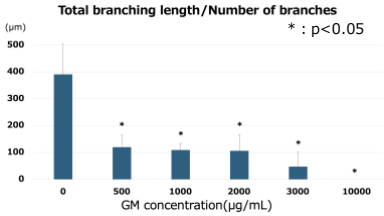

Fig.3b