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Antiproliferative and Antibacterial Effects of Pyroglutamic Acid Isolated from *Enterococcus Faecium* (Mcc-2729)

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ABSTRACT

The pyroglutamic acid (PCA) is also known as pidolic acid, 5-oxoproline. It is produced in almost all living organisms. The compound is known for its multiple physiological functions in its free and conjugated forms. PCA is reported to be a reservoir of glutamate, osmoprotectant and also enhances the stability and induces unique functions to the proteins. The peptides containing PCA are said to cause many neurological disorders and also act as a marker for the diagnosis of these disorders. The compound with such diverse functions is studied for its antiproliferative and antimicrobial activities. It is found to exhibit antiproliferative activity against both the HeLa and K-562 cell lines. Further, PCA has shown broad spectrum antibacterial activity with higher efficacy against gram positive bacteria. This is the first report highlighting the antiproliferative role of PCA even though it is ubiquitous in distribution.

Keywords
Pyroglutamic acid, antiproliferative activity, antibacterial activity, Enterococcus faecium

INTRODUCTION

Pyroglutamic acid is a derivative of glutamic acid where the amino group cyclizes to form a lactam. It is ubiquitous in distribution and found in bacteria, plants and animals (Kumar and Bachhawat 2012). This compound with such wide distribution is reported of diverse physiological functions. PCA is present in conjugated and free forms (Kumar and Bachhawat 2012). The conjugated form of PCA is reported at the N- terminal end of proteins containing glutamine or glutamate (Schilling et al 2004). This provides stability and unique functionality to the proteins. Therefore, it generated resistance against the degradation induced by aminopeptidases. Many antibodies and structural proteins like collagen, fibrinogen and fibrin are found to have PCA at the N-terminal end, thus, enhancing their half-life (Fietzek et al 1974). The n-terminal PCA in MCP-2 prevents protein degradation by proteases (Kumar and Bachhawat 2012). The specificity and activity of proteins are also determined by the presence of PCA. The PCA in thyrotropin releasing hormone is involved in the interaction with its receptor and any change in the lactam ring of PCA drastically affected the functionality of the hormone. It is known to have a similar effect on many neuropeptides (Carraway et al 1975).

The role of the free form of PCA is not substantially studied and has many lacunae. The reports mostly suggest the free PCA as a precursor and reservoir of glutamate (Kumar and Bachhawat 2012). In some halophytic and thermophilic bacteria, it is found in high concentrations possibly playing the role of osmoprotectant (Trotsenko and Khelenina 2002). Equally, it is reported to be present in high concentrations in human skin and functions as a moisturizer. PCA is also induced under osmotic stress thus playing a crucial role not only in regular physiology but also under stressed conditions. Further, PCA is also known to exhibit antidiabetic effect by modulating glucose and lipid metabolism (Yoshinari et al 2011). Inflammation is a common factor in any stress or disorder in animals. The PCA is highly induced and commonly present in diverse stress conditions of many organisms. Thus, keeping in view its ubiquitous distribution and presence in

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normal as well as stressed conditions, we tried to investigate the impact of PCA in cancerous condition. Cancer is a condition in the human body where the cells are shifted from normal physiology to abnormal physiology leading to severe stress condition. Most of the antineoplastic drugs are either natural products or their derivatives (Karikas, 2010). Moreover, the PCA is orally and intravenously active and also could cross the blood-brain barrier giving it the advantage of reaching all parts of the body with any method of administration. PCA was not studied till date for its antiproliferative activity. We also have studied the antimicrobial activity of this compound. To evaluate the effect of PCA we have opted for a more aggressive cervical cancer cell lines and leukemia cell lines namely HeLa and K 562.

MATERIALS AND METHODS

Production of Pyroglutamic acid

Enterococcus faecium (MCC-2729) was isolated from the dairy soil sample where the milk and milk product remnants are dumped over two decades. Keeping the uniqueness, nutritional richness of the source, the bacterial supernatant fractions were further investigated for their antimicrobial potential against various pathogenic organisms (Lalam et al 2015a). The cultural parameters were optimized for maximum production of the compound in the fractions. The compound was identified to be pyroglutamic acid which is presently being studied for its antiproliferative activity.

Cell culture:

Human cancer cell lines (HeLa and K562) were selected for the study. They were obtained from NCCS, Pune. Minimal essential medium containing all essential nutrients like fetal bovine serum, L-glutamine and glucose at 5%, 2mM and 4.5 g/L, respectively was used for the growth of cell lines. The conditions for growth are 37°C, 5% CO_2, 48h.

MTT assay:

The trypsinized cells of 10,000 cells/well with growth medium were taken. The culture conditions are 37°C and 5% carbondioxide. Once the cell lines attained 60-70% of confluence, the PCA was added in respective concentrations of 12.5, 25, 50, 100 and 200 µg/ml. The triplicates of each concentration were setup and cultured for 48hr. The PCA stock of 1.0mg/ml concentration was prepared in PBS. The controls for the study are culture medium and solvent. A fresh MTT stock of 5mg/ml concentration was prepared in PBS and 20µl was added in each well. The cells were incubated for 4h at 37°C. After discarding the supernatant growth medium the cells were carefully washed with PBS. 100µl of DMSO was added to dissolve the formazan product formed. The absorbance (OD) values were read at 570 nm after 30 min of incubation on an Anthos 2020 microplate reader.

Determination of antimicrobial activity and minimum inhibitory concentration

Agar well diffusion assay and broth dilution assay were performed to determine the antimicrobial potential and the minimum inhibitory concentration (MIC) of PCA against the test organisms. PCA was added to the nutrient broth to attain the desired concentrations. The broths were inoculated with the respective test organisms (*S.aureus*(MTCC 3160), *B. cereus* (MTCC 430), *B.subtilis*(MTCC 441) ,*P. aeruginosa*(MTCC 424), *E. coli* (MTCC 443)) and incubated at 37^{o}C.

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Statistical analysis:
The experiments were conducted three times. The data were analyzed by one way ANOVA. The mean values thus obtained were assessed by Dunken Multiple Range Test at $p<0.05$.

RESULTS and DISCUSSION

PCA exhibited anti-proliferative activity on both the HeLa (cervical cancer cell line) and K562 (leukemia cell line). The compound showed marginally enhanced activity against HeLa over K-562. The percentage of inhibitory activity for the HeLa and K562 were 82.41% and 60.32%, respectively at 100µg/ml concentration. The IC_{50} value for HeLa and K-562 cell lines was 60.32 µg/mL and 82.41 µg/mL respectively (Fig. 1). PCA also caused the cell aggregation and cell sinkage and most of the cells contain uncharacterized bodies, this may be due to the induction of apoptosis by the compound (Fig. 2 & 3). These results suggest that the compound PCA can be used as a lead molecule for the development of more effective derivatives.
The antimicrobial activity of PCA was earlier reported in Urabanek et al 2012. Here we again studied along with *E.coli* and extended the study to MIC values which were not reported in the earlier work. PCA showed antibacterial activity against all the tested bacteria. The MIC of PCA against the bacteria was identified to be ranged between 10µg/mL to 24µg/mL. The MIC values of *B.subtilis, S.aureus*and*B.cereus*were 10 µg/ml, 12µg/ml, and 14 µg/ml, respectively whereas the MIC values of *P.aeruginosa*is 20µg/ml and *E.coli* is 24µg/ml. There is a clear variation in the activity of PCA against gram positive and gram negative bacteria. The MIC values for the compound are provided in Table 1. This study affirms the antibacterial activity of PCA and supports the results of Urabanek et al 2012. Overall results indicate that the PCA is having higher bactericidal efficiency on gram-positive bacteria.
The antiproliferative as well as antibacterial properties establish that PCA plays a significant role in protecting the human system. The role of PCA beyond its physiological roles is very much evident with this investigation. However, the cellular role of PCA is not limited to these results and it might have various influences and functions on the living systems. The compound being predominantly biological in origin can also be chemically synthesized in easy methods and can be a safe drug or lead molecule.

CONCLUSION

The PCA exhibits antiproliferative activity on HeLa as well as K562 cell lines. It is affirmed that the inhibitory effect of PCA was higher on HeLa cell line than that of the K562 cell line. PCA was effective against both gram-positive and gram-negative bacteria with higher efficacy against gram-positive bacteria.

Acknowledgement
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Figures and Tables

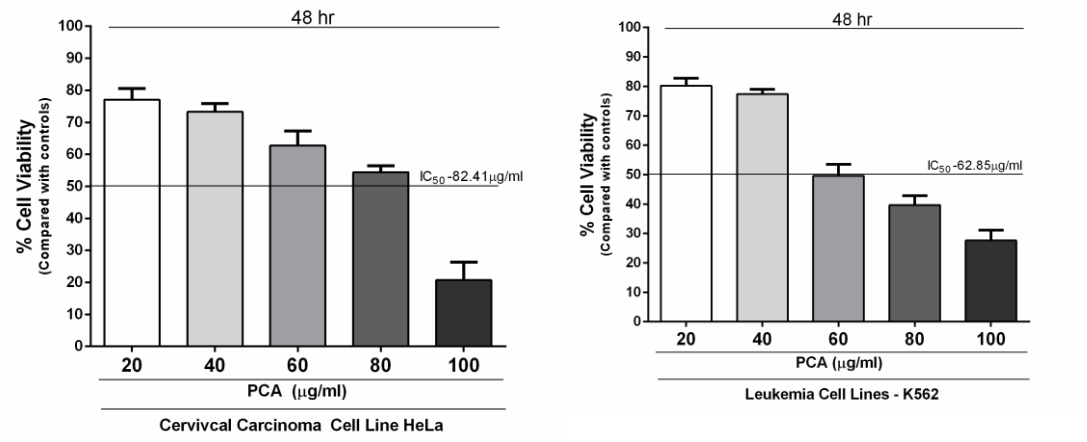

Fig 1: Inhibitory activity of PCA on HeLa and K-562 cell lines

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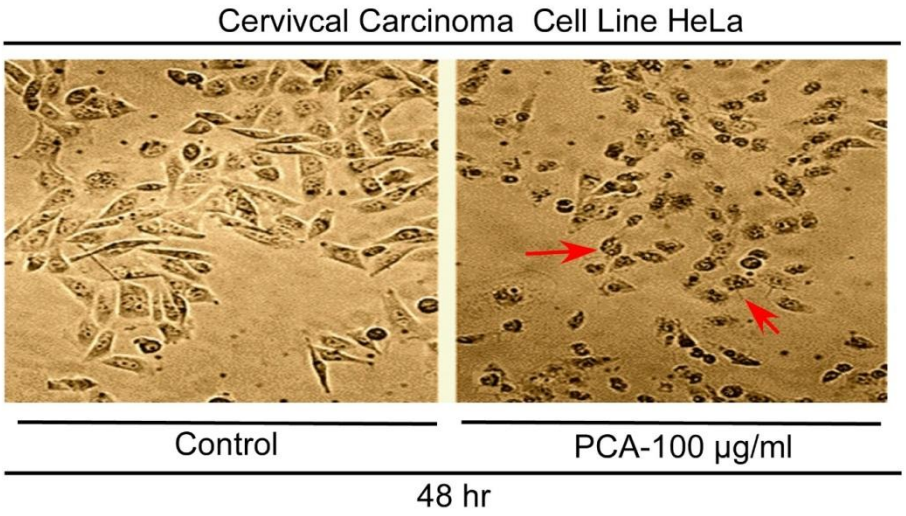

Fig 2: The impact of PCA on cervical cancer cell lines (HeLa)

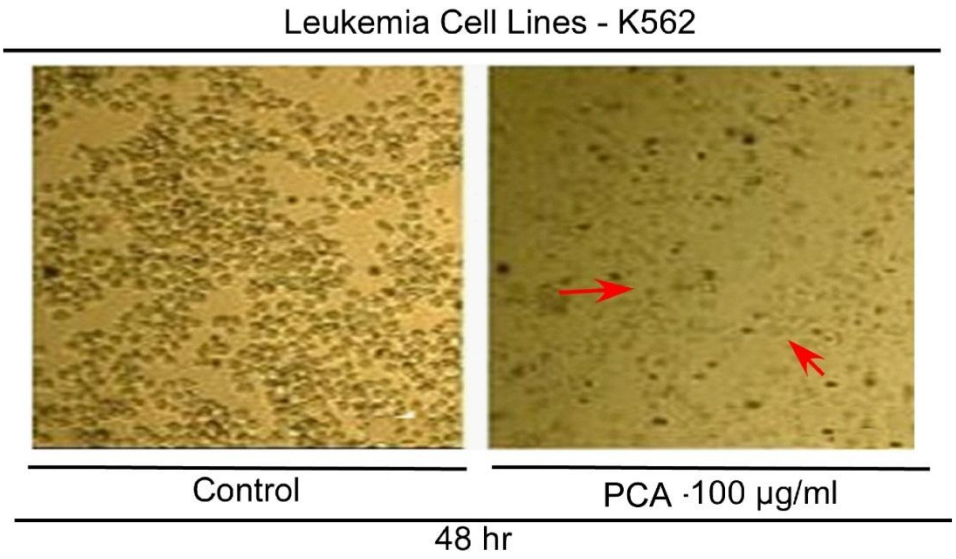

Fig 3: The impact of PCA on Leukemia cell lines (K562).

Table 1: The minimum inhibitory concentrations of PCA

Organism	MIC (µg/ml)
S.aureus(MTCC 3160)	12 ± 0.266
B. cereus (MTCC 430)	14 ± 0.232
B.Subtilis(MTCC 441)	10 ±0.614
P. aeruginosa(MTCC 424)	20 ± 0.577
E. coli (MTCC 443)	24 ± 0.333