INTRODUCTION: Since the first liver transplantation (LT) was performed in 1963, there has been much progress in surgical technique, perioperative treatment and immunosuppressive therapy. As well as the incidence of early complications decreasing steadily, such long-term complication as de novo cancer still represents a big issue. The leading reason of de novo cancer is life-long immunosuppressive therapy, which makes more than 20% of deaths during long-term follow up. The risk of de novo malignancy after LT has been reported to range from 4% to 16%.

The most frequent malignancies after LT in Korea are stomach cancer and colon cancer in men and breast cancer in women, with a relative risk >10-fold higher compared with the Korean general population. [Park HW, et al. Transplant Proc. 2012].

Considering this situation, we aimed to find a better combination of immunosuppressants for de novo colon cancer patients after liver transplantation.

MATERIAL:
• HT29, SW620, and HCT116 cell lines were used in in vitro studies
• HT29 colon cancer cell line was also used in BALB/c-nude mice animal models

METHODS:
1) MTT-assay – for cell viability
2) Western Blotting – for mTOR proteins extraction

HT29 cells were separated into 8 treatment groups: Control, Sirolimus only (5ng/ml), Tacrolimus only (5ng/ml), MMF only (500ng/ml), Metformin only (5mM), Sirolimus+Tacrolimus, and Sirolimus+MMF, Metformin+Sirolimus.

RESULTS: The strongest synergistic effect in inhibition of cell viability at MTT-assay belongs to combination of Sirolimus+Metformin – 34.2%.

The same synergistic effect of Sirolimus and Metformin combination was revealed by Western Blot on expression of proteins. In case of p70S6K the combination of Sirolimus+Metformin made – 59.7%. The expression of p4EBP1 protein was not significantly differ the combination of Sirolimus+Metformin inhibited expression as 63.3%.

CONCLUSION:

For de novo colon cancer after LT, conventional CNI immunosuppressive treatment could be effectively changed to mTOR-inhibitors in combination with metformin. This combination could be optimal in reducing tumor growth, apoptosis, and metastasis.

Our study is the first to evaluate anti-tumor effect of various IS with or without metformin on three different colon cancer cell lines. We found that a combination of Met/S showed the best anti-tumor effect on colon cancer cell lines. In in vitro experiment, metformin and sirolimus showed significant and synergistic effect in suppressing cell viability and inhibiting the expression levels of m-TOR pathway related, apoptosis related, and EMT related proteins in all three colon cancer cell lines (HT29, SW620, and HCT116). The combination of metformin and CNI (tacrolimus, cyclosporin A) failed to show similar synergism. We observed similar results in in vivo experiment. The per os treatment with the combination of metformin and sirolimus for 4 weeks dramatically reduced tumor growth likely via inhibiting mTOR pathway proteins and apoptosis related proteins.

In this study, we found that sirolimus had significant anti-tumor effect on colon cancer cell line. Furthermore, our results demonstrated both efficacy and potential benefits of the combination of sirolimus and metformin in inhibiting colon cancer. Thus,

Sirolimus and Metformin Synergistically Inhibits Colon Cancer In Vitro and In Vivo

Nadiar Mussin,
Kwang-Woong Lee, Seung Cheol Oh, Kaliyev A.A., Sultangereyev Y.
Aktobe Medical Center, Aktobe city, Kazakhstan
Department of Surgery, Seoul National University College of Medicine, Seoul, Republic of Korea