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Glycine inhibits colorectal cancer growth via suppression of VEGF induced angiogenesis

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Angiogenesis plays a pivotal role in the growth and progression of cancer. Glycine is a non-toxic amino acid with suspected anti-angiogenic effects. This study was designed to evaluate possible anti-angiogenic effects of glycine in colorectal cancer (CRC).

In vivo: The WAG-Rij/CC-531 model of metastatic CRC was used. 1 x 10^6 CC-531 tumor cells were injected subcutaneously and WAG/Rij rats were fed control or 5% glycine diets. Eight days after CRC inoculation tumor volume was significantly blunted in the glycine-fed animals (143 ± 11 mm^3) compared to the control animals (227 ± 14 mm^3; $p < 0.01$). The mean explanted tumor weight was lower in the glycine-fed animals (0.49 ± 0.09g in glycine group and 0.74 ± 0.19g in control group) and demonstrated a significant reduction of 33.8% ($p = 0.03$). In addition, microvessel density in the surrounding tumor tissue was significant reduced by 55% ($p = 0.04$).

In vitro: Further, the direct effect of glycine on human CRC cell lines HCT-116, HT-29 was assessed and different angiogenesis assays were performed to determine glycine activity on human umbilical vein endothelial cell (HUVEC). Additionally, glycine activity in inhibiting the proangiogenic effect of vascular endothelial growth factor (VEGF), as well as the conditioned medium from CRC cells was determined. Neither CRC cell growth, nor HUVEC angiogenesis assays in basal conditions were effected by glycine. However, pre-treatment with VEGF prior to glycine treatment significantly enhanced proliferation, migration, and capillary formation of HUVEC to up to 267%. Glycine neutralized this effect. HUVEC growth was inhibited in a

concentration-dependent manner, by 43.3 to 92.1% at 0.01 and 1 mM ($p = 0.001$) compared with VEGF stimulated control group. 1 mM of glycine with VEGF pretreatment reduced HUVEC migrated surface coverage by 30.0% ($p = 0.012$) and surface coverage rate was decreased from 3.9 ± 0.8 x 104 $\mu m^2/h$ to 2.8 ± 0.9 x104 $\mu m^2/h$ ($p = 0.012$). The presence of 1 mM glycine in 3D sprouting angiogenesis assay significantly decreased the cumulative sprout length to 33% ($p = 0.01$) in HUVEC pretreated with VEGF, whereas no effect was observed in the cells that did not undergo VEGF-pretreatment. Additionally, a conditioned media from VEGF overexpressing CRC cells (HCT-116 and HT-29) were angiogenically active and glycine counteracted this effect to 34 and 28% ($p < 0.05$), respectively. Further, the HUVEC lysates expressed the proteins of 48 kDa molecular weight, representing glycine receptor's (GlyR) α1 and α2 subunits. GlyR was also visualized in endothelial cells using double immunofluorescence. To determine whether the mechanism by which glycine effects VEGF induced angiogenesis involves GlyR, 50 µM of strychnine, the antagonist of the GlyR, was added to the migration and sprouting assays. Indeed, strychnine significantly blunted glycine anti-angiogenic activity in HUVEC migration by 71.1% and in capillary formation by 63.4%.

In this study, a direct effect of orally administered glycine on angiogenesis has been demonstrated in an *in vivo* model of CRC for the first time. In *in vitro*, a GlyR-mediated counteractive effect on endothelial cells after stimulation with either VEGF or CRC-conditioned culture media could be shown. Taken together, the effects described in this study could lead to future clinical trials with glycine as a cheap, easily available addition to conventional and targeted therapies against highly vascularized, metastatic tumors.