Title	Change in endogenous nitric oxide production in the healing of gastric ulcer induced by acetic acid
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S-P-3

Analysis of Erythroid Differentiation Factor in Anemia and Leukemia Based on a Combined Experimental and Computational Method

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In this project, we have identified a human DNA sequence of 288 base pairs, which expresses a peptide of 11kD containing 96 amino acids, using standard molecular cloning techniques. This peptide is called erythroid differentiation factor (EDF) which was found, according to our *in vitro* and *in vivo* experimental results, to inhibit the proliferation of murine erythroleukemia (MEL) cells and induce the cell terminal differentiation. More precisely, using MEL cell-induced leukemia BALB/c mouse model, we have shown that the EDF is able to inhibit the growth of MEL cells into tumors *in Vivo* and that the EDF-treated mice had prolonged life spans. In anemia study, using phenylhydrazin and excessive bleeding to induce anemic mouse models, we have shown that EDF is able to induce RBCs terminal differentiation with an increase in haematocrit. We then generated a 3-D molecular structure of EDF by computation methods using internet bioinformatic database facilities. We have found a single base pair mutation of G to A leading to a change of amino acid Glu to Lys at the 30th position, resulting a significant change in electrostatic potential, effective molecular surface charge distribution and conformation of EDF. Such combined experimental and computation analyses lead to better understanding the irregularity of cell differentiation in anemia and leukemia, as well as providing a powerful technique in the design of relevant protein-drugs. The methodology applies to other diseases also.

S-P-4

Change in Endogenous Nitric Oxide Production in the Healing of Gastric Ulcer Induced by Acetic Acid

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Background: Nitric oxide (NO), a short-lived free radical, plays a significant role in numerous physiological and pathological functions. It is known that both Ca^{2+}-dependent constitutive nitric oxide synthase and Ca^{2+}-independent inducible nitric oxide synthase have been detected in gastric mucosal tissues. In this study, the change in nitric oxide production in rats during ulcer healing was measured by chemiluminesence and a spin-trapping method with the use of X-band electron paramagnetic resonance (EPR) spectroscopy.

Method: Gastric ulcer was induced by applying acetic acid to the luminal surfaces in rats. One, three, five and eight days after, sera were collected and analyzed by a nitric oxide analyzer employing chemiluminesence detection. Both ulcer and normal tissues were analyzed by EPR spectroscopy and immunohistochemistry. Rats were given a nitric oxide spin trapper (DETC and ferrous sulfate) thirty minutes prior to laparotomy.

Results: The Mean (S.D.) value of nitric oxide production as measured by EPR was significantly higher on Day 1 in ulcer tissue ((27.04 (9.39)units) than normal tissue ((2.56 (0.77)units) ($p<0.005$ for Day1, $p<0.05$ for Day 3, 5 and 8) The NO_x concentration as measured by nitric oxide analyzer reached maximum value on Day 3 ((34.62 (17.89)μM) and was significantly higher than the concentrations on Days 1, 5 and 8 ($p<0.05$). The expression of iNOS reached a maximum at Day 3 ((16.28(1.95))a.u.) and decreased to minimum at Day 8 ((4.62 (3.15)a.u.)) ($p<0.05$) while the expression of ecNOS had insignificant reduction overtime.

Conclusion: The generation of nitric oxide can be directly measured by electron paramagnetic resonance spectrometer. High level of nitric oxide production at the early healing phase may be related to the severity of the ulcer since it decreases to the basal level in the late healing stage. The major production of NO appears to be associated with the iNOS expression rather than ecNOS expression.