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THESIS for Ph.D.

EFFECT OF ANTIFUNGAL AZOLE-DERIVATIVES AND CYTOKINES ON COLONY FORMATION BY NORMAL AND LEUKEMIC HEMATOPOIETIC CELLS

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SUMMARY

Myelotoxicity of therapeutic drugs may be particularly important in patients with neutropenia, since even a minor myelotoxicity may worsen their prognosis by inhibiting recovery of the bone marrow. In addition to antifungal drugs, this may be valid also for inhibitors of â-lactamase, which are used to protect â-lactam antibiotics. These latter have been demonstrated to cause neutropenia if given at high doses for prolonged periods. Recovery of the bone marrow from damage may be accelerated by some myelotropic or pleiotropic cytokines. There is, however, some concern about the use of these agents in hematological malignancies, as they may be capable of stimulating the malignant clone. There are contradictory data in the literature as for such an unwanted effect is produced by G-CSF, GM-CSF or SCF also in patients with *lymphoid* leukemia.

Our new results:

1. **Effect of antifungal drugs on colony formation by granulocyte-macrophage colony forming units (CFU- GM)**
   - 1.1. The eight azole antifungal agents studied inhibited colony formation by CFU-GM *in vitro* with the exception of fluconazole, which failed to reduce colony formation considerably even at concentrations surpassing the plasma level observed after the highest therapeutic doses.
   - 1.2. The groups of imidazoles and triazoles exhibited no separate ranges of IC_{50}s; rather, the IC_{50}s of triazoles encompassed those of the imidazoles. The order of decreasing potency (increasing molar IC_{50}) in human bone marrow cultures was itraconazole > saperconazole > clotrimazole > ketoconazole > miconazole > econazole > oxiconazole > fluconazole-b-methyl-acrylate > fluconazole-diethyl-acetate > fluconazole-â-phenyl-propionate >> fluconazole.
   - 1.3. There was a correlation between the logIC50 and logP values of the compounds studied, indicating a possible role for lipid solubility of these drugs in their capabilities of inhibiting colony formation.
   - 1.4. There was a close correlation between the murine and human $\log IC_{50}$ values of the drugs suggesting that cultures of murine bone marrow may be suitable to predict the in-vitro toxicity of azole antifungals to human CFU-GM.

2. **Effect of some ã-lactone derivatives with -lactamase inhibitory effects on colony formation by CFU-GM**
   Colony formation by CFU-GM was not considerably inhibited by two ã-lactone derivatives with â-lactamase inhibitory effects, being less toxic than their mother compound in this respect.

3. **Effect of G-CSF, GM-CSF and SCF on colony formation by leukemic blasts of patients with acute lymphoid leukemia**
   - 3.1. Contrary to their known physiologic roles, the above myelotropic and pleiotropic cytokines stimulated the formation of colonies by pediatric ALL blasts *in vitro*. Of these, GM-CSF was the least effective, whereas G-CSF and SCF stimulated similar number of colonies.
   - 3.2. The cytokine-sensitivity as well as the maximum response to the above stimuli was highly variable in the 13 fresh ALL patients studied in five years.
   - 3.3. Bone marrow samples producing spontaneous growth responded to each of the stimuli with higher numbers of colonies than cultures forming no colonies without the addition of exogenous stimuli.
   - 3.4. Combinations of G-CSF, GM-CSF and SCF were capable of stimulating the growth of colonies even in the cases not responding to single cytokines, in all cases the triple combination producing the highest number of colonies.
   - 3.5. This phenomenon may present a potential hazard to children with ALL while on adjuvant therapy with hematopoietic growth factors. *In vitro* colony assays performed prior to or in parallel with the administration of hematopoietic growth factors to ALL patients may help to forecast their possible effects on leukemic cells *in vivo*.

Thesis based on the following publications

01. **Benkõ I.,** Megyeri A., Hernádi F., Kovács P.
Antifungális hatású imidazol-származékok hatása egér csontvelõsejtek *in vitro* kolóniaképzésére.
Effect of imidazole antifungal agents on murine CFUc of bone marrow.
Acta Pharm. Hung. 66, 241-245, 1996.

02. **Benkõ, I.,** F. Hernádi, A. Megyeri, A. Kiss, G. Somogyi, Z. Tegyey, F. Kraicsovits, P. Kovács,
Comparison of toxicity of fluconazole and other azole antifungal drugs to murine and human granulocyte-macrophage progenitor cells (CFU-GM) *in vitro*.
J.Antimicrob.Chemother. 43, 675-681, 1999.
IF: 3,296

03. Gál, Zs., Á. Koncz, I. Szabó, E. Deák, **I. Benkõ,** Gy. Barabás, F. Hernádi, P. Kovács, A synthetic γ-lactone group with β-lactamase inhibitory and sporulation effects.
J. Chemother. 12, 274-279, 2000.
IF: 0,921

04. **Benkõ, I.,** P. Kovács, I. Szegedi, A. Megyeri, A. Kiss, É. Oláh, J. Kappelmayer, C. Kiss, Effect of myelopoietic and pleiotropic cytokines on colony formation of blast cells of children with acute lymphoblastic leukemia.
Naunyn Schmiedeberg's Arch. Pharmacol. 363, 499-508 2001.
IF: 2,869

Other publications

01. Mórocz, I., **I. Benkõ,** C. Kiss, Z. Bársony, Focal nodular hyperplasia of the liver after clomiphen treatment in a young boy. Acta Paediatr. Hung. 27, 15-21, 1986.

02. Kovács, P., F. Hernádi, L. Institóris, **I. Benkõ**, Early regeneration of Colony Forming Units in culture (CFUc) of murine bone marrow after moderate doses of dibromomannitol. Acta Physiol. Hung. 75, 183-184, 1990.

03. Kovács, P., I**. Benkõ**, F. Hernádi, A. Jeney, J. Kralovánszky, Effect of combinations of fluorouracil, uridine and leucovorin on murine bone marrow "Colony Forming Units in culture" (CFUc). Pharmacol. Res. 25, 308-309, 1992.
IF: 0,702

04. Hernádi, F., F. Krajcsovics, I. Czink, Á. Koncz, T. Schmidt, M. Fuxreiter, **I. Benkõ**, P. Kovács, Investigation of antifungal effects of fluconazole ester derivatives. J. Chemother. 5 , 430-431, 1993.
IF: 0,921

05. Kovács, P., F. Hernádi, E. Csányi, E. Király, **I. Benkõ**, Effect of 1,6-di-o-methylsulfonyl-3,4-di-o-methyl-d-mannitol (GYKI-17230) on murine bone marrow colony forming units (CFUc). J. Chemother. 5 , 804-805, 1993.
IF: 0,921

06. **Benkõ, I.,** L. Maródi, A. Megyeri, R. Káposzta, P. Kovács, Effect of granulocyte colony-stimulating factor on granulopoiesis of congenital neutropenic children. Acta Physiol. Hung. 84, 169-170, 1996.

07. Megyeri, A., **I. Benkõ,** A. Jeney, J. Kralovánszky, P. Kovács, Effect of ethyldeoxyuridine on 5-fluorouracil-induced neutropenia. Acta Physiol. Hung. 84. 217-218, 1996.

08. Kiss, C., **Benkõ I**, Szegedi I, Balogh E, Kovács P, Oláh É: Myelodysplastic syndromes in children: Clinical and laboratory observations from a single center in North-East Hungary. Med. Pediatr. Oncol. (submitted)

IF: 1,518

09. Kovács, P., **I. Benkõ,** F. Hernádi, L. Institóris, Damage and regeneration of bone marrow in mice given single or repeated doses of disuccinyl-dianhydrogalactitol (DisuDAG). In: Recent Advances in Chemotherapy, Adam, D., Büchner T., Rubinstein, E., Eds., Futuramed Verlagsges. pp. 2630-2631, Munich 1992.

10. Kovács, P**., I. Benkõ**, A. Megyeri, F. Hernádi, A. Jeney, J. Kralovánszky, Effect of Uridine Rescue on Fluorouracil-Induced Neutropenia. Recent Advances in Chemotherapy, J. Einhorn, C.E. Nord, S.R. Norrby, Eds., American Society for Microbiology, p. 952, Washington, D.C. 1994.

11. **Benkõ, I.,** P. Kovács, L. Maródi, F. Hernádi, Granulocyte-Macrophage CFUc Assay in Congenital Neutropenic Children Treated with Granulocyte Colony-Stimulating Factor. Recent Advances in Chemotherapy, J. Einhorn, C.E. Nord, S.R. Norrby, Eds., American Society for Microbiology, p. 994, Washington, D.C. 1994.