10 Pardee, A.B. and James, L.J. (1975) Selective killing of transformed baby hamster kidney (BHK) cells. *Proc. Natl. Acad. Sci. U. S. A.* 72, 4994–4998
11 Blagosklonny, M.V. and Darzynkiewicz, Z. (2002) Cyclotherapy: protection of normal cells and unshielding of cancer cells. *Cell Cycle* 1, 375–382
12 Rowinsky, E.K. and Donehower, R.C. (1995) Paclitaxel (Taxol). *New Engl. J. Med.* 332, 1004–1014
13 Abal, M. *et al.* (2003) Taxanes: microtubule and centrosome targets, and cell cycle dependent mechanisms of action. *Curr. Cancer Drug Targets* 3, 193–203
14 Blagosklonny, M.V. (2002) Sequential activation and inactivation of G2 checkpoints for selective killing of p53-deficient cells by microtubule-active drugs. *Oncogene* 21, 6249–6254
15 Rahmani, M. and Grant, S. (2002) UCN-01 (7-hydroxystauorsporine) blocks PMA-induced maturation and reciprocally promotes apoptosis in human myelomonocytic leukemia cells (U937). *Cell Cycle* 1, 273–281
16 Blagosklonny, M.V. (2003) A new science-business paradigm in anticancer drug development. *Trends Biotechnol.* 21, 103–106
17 Lane, D.P. and Hupp, T.R. (2003) Drug discovery and p53. *Drug Discov. Today* 8, 347–355
18 Los, M. *et al.* (2003) Anticancer drugs of tomorrow: apoptotic pathways as targets for drug design. *Drug Discov. Today* 8, 67–77
19 Zhivotovsky, B. (2003) More than one road to kill tumor cells: why are they not always successful? *Cell Cycle* 2, 31–33
20 Reed, J.C. (2002) Apoptosis-based therapies. *Nat. Rev. Drug Discov.* 1, 111–121
21 Reed, J.C. (2003) Apoptosis-targeted therapies for cancer. *Cancer Cell* 3, 17–22
22 Makin, G. and Dive, C. (2003) Recent advances in understanding apoptosis: new therapeutic opportunities in cancer chemotherapy. *Trends Mol. Med.* 9, 251–255
23 Blagosklonny, M.V. (2001) Treatment with inhibitors of caspases, that are substrates of drug transporters, selectively permits chemotherapy-induced apoptosis in multidrug-resistant cells but protects normal cells. *Leukemia* 15, 936–941
24 Bunz, F. *et al.* (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* 282, 1497–1501

The *Discussion Forum* provides a medium for airing your views on any issues related to the pharmaceutical industry and obtaining feedback and discussion on these views from others in the field. You can discuss issues that get you hot under the collar, practical problems at the bench, recently published literature, or just something bizarre or humorous that you wish to share. Publication of letters in this section is subject to editorial discretion and company-promotional letters will be rejected immediately. Furthermore, the views provided are those of the authors and are not intended to represent the views of the companies they work for. Moreover, these views do not reflect those of Elsevier, *Drug Discovery Today* or its editorial team. Please submit all letters to Steve Carney, Editor, *Drug Discovery Today*, e-mail: S.Carney@elsevier.com

Probiotics and prebiotics: why should the medical community pay attention?

In a recent issue of *Drug Discovery Today*, Tuohy and colleagues [1] presented a review article on the use of probiotics and prebiotics for improved gut health. Such a review raises two questions. First, why is there such a topic in a journal dedicated to 'drug discovery'? Second, is it justified to inform the medical community about scientific data on the effects of probiotics and prebiotics? Probiotics and prebiotics are classified as 'functional food ingredients' or 'food ingredients for which it can be scientifically demonstrated that they beneficially affect functions in the body relevant to well being and health or to the reduction of risk of disease' [2]. Thus they belong to nutrition not to pharmacology; they are foods, not drugs.

However, publishing the review was justified. Indeed, the primary target of probiotics and prebiotics is intestinal microflora – the large population of bacteria that colonize the large bowel and play a key role in human well being and health. The importance of the colonic microflora, particularly its composition, has largely been underestimated in medicine mainly because quantitative analysis was difficult owing to methodological problems associated with identifying, culturing and counting anaerobes. However, molecular approaches are now available that overcome these limitations and more data are now accumulating that demonstrate the key roles of some populations of bacteria, the so-called 'health promoting' bacteria, in well being and health [3]. In addition, the medical community has shown an increased interest for these products.

The review by Tuohy and colleagues is an excellent update of the data presently available. The existence of probiotics has been known for more than a century and data already exist that show beneficial effects in many medical conditions such as diarrhoea, intestinal infections and intestinal inflammation. The concept of prebiotics is more recent [4] but convincing experimental data do exist and human studies are ongoing to test different hypotheses in medically relevant situations and these show great promise. In addition, the synbiotic approach of combining both probiotics and prebiotics is attracting more and more interest.

1359-6446/03/$ – see front matter ©2003 Elsevier Science Ltd. All rights reserved. PII: S1359-6446(03)02844-7

The mechanism of action of probiotics and prebiotics is likely to involve modulation of key body functions relevant to the prevention or management of disease-prone situations. The first target of these effects is the intestinal microflora. However, according to more recent research, they may also modulate immune functions, in addition to essential regulatory processes, particularly those mediated by gastrointestinal peptides [5].

Probiotics and prebiotics sit at the interface between nutrition and pharmacology. They are currently predominantly used in foods, but tomorrow they shall find their place in medicine, even if they will probably never be therapeutic drugs.

Drug Discovery Today aims to bring to the attention of the medical community new developments that help patient care. In that context, a review on probiotics and prebiotics is important to inform, to stimulate interest, to create opportunities for further research and to hopefully help patients.

References

1 Tuohy, K.M. *et al.* (2003) Using probiotics and prebiotics to improve gut health. *Drug Discov. Today* 8, 692–700

2 Diplock, A.T. *et al.* (1999) Scientific concepts of functional foods in Europe: consensus document. *Br. J. Nutr.* 81, Suppl. 1, S1–S28

3 Delzenne, N.M. *et al.* (2002) Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. *Br. J. Nutr.* 87, Suppl. 2, S255–S259

4 Gibson, G.R. and Roberfroid, M.B. (1995) Dietary modulation of the human colonic microflora: introducing the concept of prebiotic. *J. Nutr.* 125, 1401–1412

5 Teitelbaum, J.E. and Walker, W.A. (2002) Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. *Ann. Rev. Nutr.* 22, 107–138

Marcel Roberfroid
Emeritus Professor
Université Catholique de Louvain
7A Rue du Rondia
B-1348 Louvain-La-Neuve
Belgium

Ligand-selective signaling and high-content screening for GPCR drugs

Intracellular signals that are observed following stimulation of G protein-coupled receptors (GPCRs) by synthetic ligands are sometimes unpredictable. Hence, as elegantly delineated in the recent review by Graham Milligan [1] and the subsequent update article by Terry Kenakin [2], GPCRs-directed drug screening should not rely on a single assay, because lack of efficacy for a certain biochemical event does not necessarily indicate lack of receptor activation. Rather, drug-screening protocols should employ a multitude of biochemical events, including measurements of different second messengers, downstream events, receptor internalization and related gene transcription. Employing such high-content screening (HCS) protocols should enable better identification of promising GPCR compounds in drug development projects.

We would like to illustrate this point by presenting our decade-old experience with the identification of AF102B (Cevimeline) as a selective M1 agonist with a unique signaling profile [3,4], long before HCS platforms became available. When assayed in Chinese hamster ovary (CHO) fibroblasts stably transfected with the M1 muscarinic receptor gene, AF102B behaved as a classical M1 antagonist when measuring adenylate cyclase, fully blocking its activation by the non-selective cholinergic agonist carbachol [4].

However, it was soon realized that AF102B was far from being an antagonist. When measuring activation of phosphatidyl-inositol specific phospholipase C or phopsholipase A2 in the same cell line, AF102B behaved as a partial agonist [4]. The most amazing observation, however, was from confocal microscopy imaging of intracellular free calcium ions. Here, AF102B behaved as a super-agonist, yielding a stronger rise in calcium ions than carbachol [4]. Notably, these assays were conducted using similar assay conditions in the same cells. Thus, our studies illustrated that a single ligand, by activating a particular GPCR, was capable of exhibiting a signaling profile of an antagonist, a partial-agonist, or even a super-agonist, depending on the signal being measured. Subsequent studies, which explored late events related to M1 receptor activation and using the neuronal cell line PC12M1 – such as secretion of the amyloid precursor protein [5], NGF-induced neurite outgrowth [6] or inhibition of *tau* phosphorylation [7] – indicated that, indeed, AF102B was capable of slightly surpassing the efficacy of the classical agonist carbachol in mediating certain late cellular events.

We coined the term 'ligand-selective signaling' to describe such novel observations, and suggested that they represented a universal aspect of GPCR activation [4]. Namely, we proposed that the discrete activation profiles of second-messenger signaling pathways reflected, at least in part, the capacity of rigid ligands, such as AF102B, to enable only a limited subset of ligand–receptor conformations, compared with the larger scope allowed by full agonists, which are typically more flexible molecules [4]. Our suggestion that such 'ligand-selective signaling' via M1 muscarinic receptors reflects different ligand–receptor conformations remains unproven, and should await X-ray analysis of purified M1 receptors crystallized in the presence of different ligands. However, clues for discrete ligand-dependent signaling profiles were subsequently demonstrated for other GPCRs, such as β-2 adrenergic [8] and α-2 adrenergic receptors [9].

Moreover, the realization of ligand-dictated gene transcription profiles for nuclear steroid hormone receptors led to the development of selective estrogen

 1359-6446/03/$ – see front matter ©2003 Elsevier Science Ltd. All rights reserved. PII: S1359-6446(03)02897-6