Symposium 2

dedicated to Prof. dr hab. RYSZARD BRUS

Evidence-based medicine. The progress and trap for pharmacotherapy

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Owing to a great progress in clinical chemistry connected with utilization of applied mathematics, the pharmacokinetics came into being. The previously unknown objective methods of research of drugs in humans were discovered, among them controlled clinical trials (CCT).

These new methodologies generated a new clinical discipline called clinical pharmacology (CPH) which has its roots in basic pharmacology but is applied in clinical specialties. This field is very young, and was recognized by World Health Organization in 1970.

Up till 1990s, several enthusiasts had been developing quickly CPH. The scope and development of this discipline is presented in the first part of this lecture. At the end of the 20th century, the studies of drugs performed in humans were in the centre of interest as well as an object of great pressure from pharmaceutical industry, politicians, and the public. These phenomena started to influence unfavourably CPH practiced and taught at medical university faculties and patients' care units.

Government, university authorities, non-profit organizations are not interested in supporting objective research in CPH at the highest academic level. The industry considers the mentioned studies as a treat to its profit.

CCT was developed for objective comparison of effectiveness and efficacy of an old (standard) drug with the newly approved substance.

The main purpose of this type of study is the rejection of null hypothesis.

These trials elicited in 1990s a strong movement toward evidence-based medicine. A few years ago trials were performed in independent academic centers. These studies were in experienced hands of the teams consisting of highly competent specialists of several fields of medicine.

These centers contributed to the quality, intellectual rigor and impact of such clinical trials. But as economic pressure increases, this may be a thing of the past.

Currently, pharmaceutical companies curtailed the participation of academic centers in CCT to 40%.

According to EU Parliament decision, the pharmaceutical industry adopted the whole control over CCT. Politicians and society demand the instant application of new observations and discoveries into practice.

Then, new drugs approved in 2003 are mentioned. Finally, a general proposal how to improve the status of academic CPH and creditability of CCT is suggested.

To develop basic studies on the mechanisms of action of drugs in humans, the most recognized academic experts, who understand the importance of CPH, have to discuss the necessity of funding of this type of research with university authorities, non-profit organization and Ministry of Health.

To soften unavoidable conflict of interests, a laborious discussion between academic scientists, pharmaceutical companies and governmental authorities is necessary.

There is also an urgent necessity of a new legislative acts.

These proposals are very general and very far away from perfection. They were presented here to conclude this lecture on the present status of clinical pharmacology with the statement that the real threat for this discipline exists.

Advances in dopamine research: success of three decades of research collaboration between the Medical University of Silesia in Poland and the Quillen College of Medicine at East Tennessee State University in the USA

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The dim light that shone in 1969 when Ryszard Brus and I met as colleagues in the laboratory of David M. Jacobowitz at the University of Pennsylvania, remained lit when first I visited the Medical University of Silesia in 1975 – at Ryszard's invitation. The light glimmered when we next met at International Pharmacology Meetings, and it was kept aglow when Ryszard visited my lab in 1981. By 1990 the light, nearly extinguished, began to flicker when I next visited Ryszard in Poland. Then he came to me in 1991, and by alternating visits since then, the light began to burn brightly, and it has stayed bright for the past 18 years. In our inaugural study, we produced life-long dopamine (DA) receptor supersensitivity (RSS) in rats without introducing a neurotoxin – this had not been done before. We then engaged this model to discern characteristics of DARSS. This process is now used to produce one of only a few animal models of schizophrenia. Next, we produced a new animal model of attention deficit hyperactivity disorder (ADHD), the only novel dopaminergic animal model since B. Shaywitz' model in 1967. It was this discovery that exposed the link between serotonin (5-HT) neuronal regulation of DA nerves, and demonstrated the potential usefulness of 5-HT antagonists in ADHD. Another project led to development of the only new animal model of tardive dyskinesia (TD) in the past 25 years, one more robust and more amenable to study than J. Waddington's valuable model of 1980. Our model further exposed the importance of 5-HT antagonists in regulating TD. Now from our research findings we see clear links for 5-HT in Parkinson Disease (PD). Psychiatric (schizophrenia, ADHD) and neurological (PD, TD) applications derived from our studies. Still, we continued to take the road less traveled, and engaged in *in vivo* microdialysis studies to determine even stronger associations between 5-HT and DA; and invoke reactive oxygen species as intermediaries in cellular events. Now, histaminergic and other systems are entering the arena for study. The deep friendship and rich series of scientific discoveries that mark our collaboration are part of the legacy that now thrives and which we will leave behind. We have each traveled farther than if we had each traveled alone.

Mechanism of copper-induced cell death

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Wilson's disease is a disorder with a genetic mutation in copper transporter ATPase (ATP7B), resulting in the accumulation of copper. Interestingly, a study on 282 cases of Wilson's disease, evaluated over 3 decades, revealed that 69% of patients presented neurological symptoms, where parkinsonism was predominant (62%). This means that 43% of patients with Wilson's disease have parkinsonism. Interestingly, young workers from copper smelting industry also developed parkinsonism. The questions are why copper-induced cell death is so selective in the brain resulting in parkinsonian symptoms and what is the mechanism for copper-induced cell death.

We found that $CuSO_4$ forms a complex with dopamine (CuDA) and this complex induces cell death in cells capable of dopamine uptake. This neurotoxic action of the CuDA complex was dependent on(i) the existence of dopamine uptake; (ii) dopamine oxidation to aminochrome; and (iii) inhibition of DT-diaphorase, which catalyzes two-electron reduction. $CuSO_4$ was also able to induce degeneration of dopaminergic neurons in rats with unilateral intranigral injection of 0.25 nmol $CuSO_4$ and 2 nmol dicoumarol. These animals presented a significant and characteristic contralateral rotational behavior ($p < 0.001$) when they were systemically stimulated with apomorphine (0.5 mg/kg *sc*). This effect is similar to that observed in rats injected unilaterally with 6-hydroxydopamine used as positive control. The behavioral effects correlated with the loss of tyrosine hydroxylase-positive fiber in substantia nigra and also striatum with unilateral intranigral injection with 2 nmol $CuSO_4$ together with 2 nmol dicoumarol. We investigated the mechanism of cell death induced by CuDA in a catecholaminergic cell RCSN-3. We found that CuDA induced a rapid cell death until 4 h: $65 \pm 1\%$ ($p < 0.001$) which increased in the presence of dicoumarol, an inhibitor of DT-diaphorase: $82 \pm 2\%$ ($p < 0.001$). We also noted a slower increase in the cell death until 12 h. The cell death induced by CuDA in the absence and presence of 100 μM dicoumarol is characterized by (i) chromatin alteration determined by Feulgen method; (ii) DNA condensation determined by transmission electron microscopy; (iii) DNA fragmentation determined by DNA laddering at 12 h; (iv) significant changes in the mitochondrial membrane potential determined by JC-1; (v) AIF release; (vi) formation of autophagic vacuoles at 2, 4 and 7 h and no activation of caspase 3. However, the externalization of phosphatidyl serine (63 ± 9; $p < 0.01$) induced by CuDA alone was inhibited by 5 μM cyclosporine A (72%, $p < 0.001$) while cyclosporine A had no effect on a moderate phosphatidyl serine externalization induced by CuDA in the presence of 100 μM dicoumarol.

The fact that copper neurotoxicity depends on (i) formation of dopamine complex and (ii) its uptake *via* dopamine transporters, opens a new pharmacological target in Wilson's disease to prevent parkinsonian symptoms.

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Biological effects of porphyrins: toxicological implications

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Porphyrins are compounds present in all living organisms, important for their function. They play a key role in metabolism, but also may exert toxic effects. Porphyrias, a group of metabolic disorders of hem biosynthesis, are caused in humans by inherited or rarely acquired deficiency of one of eight enzymes of this metabolic pathway. The resulting overproduction of hem precursors exerts toxic effects associated with characteristic clinical features. Among them are neurological and psychiatric symptoms, such as paresis of the lower and upper extremities, convulsions or anxiety, depression and delirium. Applications of porphyrins for cancer photodynamic therapy and for disinfection of microbiologically polluted water increases a risk of toxic effects.

The study of toxic effects of different synthetic porphyrin analogs revealed their convulsant effects in mice and rats. The mechanism of this effect is under study.

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The effects of prenatal exposure to amphetamine on electroretinogram of the rat

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We have found that gestational exposure to amphetamine (A) did not influence the FVEP of offspring Wistar rats. The aim of the present study was to examine the effect of A on retinal activity by electroretinography (ERG) in the same offspring rats. Wistar rats were examined under general anesthesia by chloral hydrate (*ip*). DTL ERG electrode was put on rat's eye along the margin of lower eyelid. A needle electrode was inserted subcutaneously in the lower lid as a reference one. Gold cup ground electrode was put on the rat's tongue. Lids were opened by single sutures. Pupil was dilated by 1% tropicamidum. Only left eye of each rat was chosen for examination. The animal was laying inside the Ganzfield stimulator. Electrophysiological equipment by LKC (USA), UTAS E-2000 program was used. ERG was examined in photopic conditions by single and flicker white flashes 5, 10, 15, 20, 25 and 30 Hz and b-wave amplitude was analyzed. Student's *t*-test and C-Cochran & Cox test were used for statistical analysis with significance $p < 0.05$. We have found that gestational exposure to amphetamine increased markedly the b-wave of ERG. After a single flash, the b-wave amplitude was in control group (C) 14.66 μV in comparison to group A 34.43 μV (244%). After a flicker mode stimulation 5, 10, 15, 20 Hz, the b-wave amplitude rose in group A in comparison to group C by 194%, 252%, 251%, 247%, respectively. The largest increase inbwave amplitude after flicker 25 Hz (903%) and 30 Hz was observed in group A. The results are open to discussion. In conclusion, gestational exposure to A increases the retina's light-sensitivity in offspring.

Therapeutic potential of nicotinic agonists in neuropsychiatric/neurodegenerative disorders

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Nicotine is a highly addictive drug with complex pharmacological actions. Some of this complexity is due to varied nicotinic receptor subtypes, their differential distribution in discrete brain regions and their relative sensitivity and desensitization following nicotine exposure. The presence of yet other nicotinic receptor subtypes in the autonomic ganglia and the neuromuscular junction can contribute to the peripheral toxicity, manifested in muscle paralysis and respiratory failure following exposure to a relatively high concentration of nicotine. On the other hand, stimulation of specific central nicotinic receptors by a relatively low concentration of nicotine may result in cognitive enhancement, mood regulation, analgesic and even neuroprotective effects. The prevalence of smoking in various neuropsychiatric disorders, particularly in schizophrenia and depression has led to the "self medication" hypothesis in these disorders. In this presentation, preclinical data in support of such hypothesis will be provided. Specifically, it will be shown that nicotine may act as an "antidepressant" or "antipsychotic" in several animal models. Moreover, data on neuroprotective effects of nicotine against various toxic insults such as alcohol or salsolinol in primary neuronal or neuroblastoma cell lines will be provided. It will be argued that nicotinic receptors may offer targets for novel drugs in the treatment of neuropsychiatric or neurodegenerative disorders.

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