SURPRISES AND OMISSIONS IN TOXICOLOGY

(HONORARY SPEAKER)

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SUMMARY

The paper describes expected and unexpected results gained from studies performed decades ago, and so to say – forgotten.

1. Different bacterial toxins can induce considerable changes in pharmacokinetics and pharmacodynamics of applied drugs. To admit clinical trials, only results from healthy human volunteers are required, however.

2. Antagonists to the toxicity of bacterial toxins in general have to be administered prior to the toxin. However, adenosine triphosphate (ATP) is effective also when applied after toxins. ATP is “in” again in contemporary research.

3. A controlled clinical trial revealed substantial differences between the D- and D,L-form of cycloserin.

4. The antimetabolite 6-azauracil riboside and eventually its triacetate derivative was claimed to possess antitumor properties. However, a controlled clinical trial did not confirm its potency in this aspect. On the other hand, the tolerance was excellent. This finding encouraged clinical trials in psoriasis, a disease of autoimmune etiology. Moreover, beneficial effects and tolerance of the compound was described in herpes zoster and even in smallpox. On the basis of these results a controlled clinical trial in rheumatoid arthritis, also judged to be an autoimmune disease, was started. Because of early high toxicity, the study was discontinued.

5. High doses of the compound induce ocular lesions in animals.

The above examples justify the title of this paper.

Key words: bacterial toxins, cycloserin, 6-azauridine

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INTRODUCTION

Pharmacology and toxicology have kept up with contemporary science. As a consequence there have been an exponential increase in the fields. The International Union of Pharmacology (IUPHAR) and the International Union of Toxicology (IUTOX), as well as their respective European counterparts, i.e. EPHAR and EUROTOX are proof of it. However, the acceleration of recent scientific information often neglects previous scientific achievements. Our experience gained successively over the last fifty years demonstrates surprises, omissions, etc. of the results of our group. This account not only reminds them but hopefully it might draw attention to neglected topics in toxicology. We will try to follow the chronology of our work.

BACTERIAL TOXINS

Thanks to Karel Raška and his coworkers, we had access to bacterial toxins. In collaboration with them, they became an important research subject for us (1, 2). Eventually, we used intoxications with several bacterial toxins to study possible changes of absorption and later also the pharmacokinetics of drugs. The different toxins induced different changes in various pharmacodynamic and pharmacokinetic parameters (3-6). Interestingly, the lethal effects of a number of toxins was diminished by adenosine triphosphate (ATP), irrespective of the time of its administration. Such experiments have either been forgotten or neglected. Unfortunately, further research had to be abandoned because of the unfavorable political climate in the early fifties.

CONTROLLED CLINICAL TRIALS

Our group was among the first on the European continent who organized controlled clinical trials. Several decades ago, the chirality of compounds was not yet in the centre of attention. D-4-amino-isoxazolidin (D-cycloserin) was introduced as a new antituberculous drug. It had some neurotoxic side effects. In our country, the D,L-form was synthesized and marketed after favourable reports in open field studies (lower neurotoxicity and equal anti-TB activity). With broader use of the D,L-form, complaints of its higher neurotoxicity occurred. Therefore, a controlled clinical trial of both types of drugs was performed, including a placebo group. For ethical reasons, the patients were concomitantly treated in the standard way (i.e. isoniazid + p-aminosalicylic acid). The results revealed significantly fewer side effects in the D-form treated patients. Thus, the results pointed out that the omission of detailed preclinical studies could result in serious clinical consequences. Later studies on animals confirmed these differences (7, 8). This is a nice example of feedback from human to animal
studies. Moreover, in the placebo group, some complaints appeared, even a rush. Their number was of course lower. Nowadays, the patient in controlled clinical trials has to be informed about possible effects and side effects. This may influence the balance between the drug and placebo effect. Fortunately, chirality is now generally considered in the development of new drugs.

6-AZAURACIL, 6-AZAURIDINE, AND 6-AZAURIDINE TRIACETATE STUDIES

This last part demonstrates the results of intensive and extensive research on the antimetabolites mentioned above. The research was performed in collaboration mainly between the Czechoslovak Academy of Sciences (Institute of Organic Chemistry and Biochemistry, and Institute of Pharmacology) and Yale University, New Haven, Connecticut. Some of the findings were first described by the American authors and later confirmed in Prague. Other results came the other way round. A symposium summarizing and discussing experimental and clinical observations obtained both in the U.S.A. and in Czechoslovakia was held in 1967 (9).

Some early findings indicated that 6-azauridine inhibited the growth of certain animal tumors. Therefore, our group performed detailed toxicological studies with 6-azauracil and 6-azauracil riboside. In these studies, toxic effects were observed. Their appearance depended on the size of the doses and the length of treatment (10). The administration of 6-azauridine was possible only intravenously. After further intensive experimental research, encouraging results in open study on humans were reported by the collaborating group in the U.S.A. A controlled clinical trial in patients with leukemia was performed in four Czech oncological centers. The results were disappointing concerning the antitumor effect. On the other hand, the drug was very well tolerated, and virtually without side effects. These results supported further research in several ways.

Psoriasis is considered to be an autoimmune disease. The necessity of parenteral administration of 6-azauridine would not be practicable. Fortunately, a suitable way to overcome this obstacle was the synthesis of 6-azauridine triacetate (azaribine). Detailed toxicological studies done in six different animal species including humans revealed marked inter-species differences in the deacetylation of the drug (11-14) (Fig. 1). Rapid deacetylation was found in rodents, intermediate in rabbits, and slow in dogs, pigs and humans. Trials in the U.S. and in Czechoslovakia confirmed the expectancies of favorable therapeutic effects, useful especially in serious cases of psoriasis. This led to investigations of the usefulness of these antimetabolite compounds in other diseases. Positive results were gained with the local administration in herpes zoster affections of the eye. Elís conducted a controlled clinical trial in the treatment of smallpox in India. Positive results, i.e. lower mortality were clear in ordinary smallpox, while the drug had no significant effect in haemorrhagic cases.

After all these attempts, rheumatoid arthritis, also considered to be of autoimmune origin, was chosen for further clinical trials. In this indication, serious side effects reminding the toxicity described originally in animal experiments occurred already after several days of the treatment. Therefore, the trial was discontinued immediately.

Fig. 1. The time course of the deacetylation of 2',3',5'-tri-O-acetyl-6-azauridine in plasma in man and six laboratory animal species. The values are expressed as the mean percentage of absorbance.

The last finding on side effects of 6-azauridine, not published as yet, came unexpectedly from experiments analyzing the genetic aspects of its lethal toxicity in mice. Several inbred strains, including those with pigmented eyes had been used in this study (15, 16). Irreversible corneal toxicity (opacities) of high doses of 6-azauridine was accidentally observed (Fig. 2). It was characterized by inflammation of the external corneal epithelium leading to
its degeneration and necrobiosis and, in the most severe cases, to panophthal-mia. The effect can be induced by topical application and can be observed in both pigmented and albino mice, as well as in rats. One of the reasons why this effect was not found before may also be the fact that as a routine, solely albino mice had been used thus far. The effect can easily be overlooked in albino animals without paying special attention to the eye. We present these findings as a tool for further research.

CONCLUSION

These examples of our research activities justify the title of this paper. This might encourage others in re-thinking their past and present approaches in their fields.

The main author (H.R.) expresses her deep gratitude not only to all co-workers quoted in this paper, but also to the long list of many others who over the past 25 years brought Czech and Slovak pharmacology and toxicology to an internationally acknowledged high standard.

REFERENCES