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The early history of intracellular cysteine proteinase inhibitors

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1. ABSTRACT

Scientists at the beginning of their careers often rely on the information obtained from review articles. Furthermore, the review articles are commonly used by sponsors in making their funding decisions of scientific projects. Thus it is very important to give a balanced view of the past as well as the present of the field discussed in those papers. In the present letter I wish to complement a review article previously published in Frontiers in Bioscience.

2. INTRODUCTION

A recent review calls for critical discussion and an analysis of the basic findings in the field of cysteine proteinase inhibitors. In an article of Turk *et al.* the most basic findings in the field were neglected (1). As a result of that, the much later article is referred to as the pioneering study in the field (2). I have worked with cysteine proteinase inhibitors almost from the very beginning of the history of these proteins. Thus, it is my duty for the future generations of scientists to share my views regarding the first steps of the inhibitor studies and fill some gaps in the information given in Turk *et al.* (1).

Objective and ethical information in review articles has recently become even more important as the funding of science in the member states of the EU has largely shifted from national to EU-based sources. It is well known that the vast number of the applications and the limited time allotted for their processing forces the board members of the EU's scientific funds to rely on review articles instead of original literature. In addition, young scientists at the beginning of their careers often rely on review articles. Therefore, it is important that the information in these review articles is as reliable as possible, and both the writer and the publisher must feel their responsibility.

3. A SHORT HISTORY OF INHIBITORS AND CORRECTIONS TO TURK *ET AL*

The Japanese scientists Tokuda, Tokaji, Udaka and Hayashi were the first to find intracellular mammalian cysteine proteinase inhibitors in the 1960's. They purified, characterized and crystallized the inhibitor from rabbit skin. They also studied the role of the inhibitor in the Arthus inflammatory reaction (3, 4, 5, 6).

My own experimental knowledge of these inhibitors stems from the year 1975 when I joined the group of Dr. Mikko Järvinen in the Department of Pathology, University of Oulu, Finland. Järvinen had recently isolated BANA hydrolase from the rat skin (7). This enzyme is nowadays known as cathepsin H (8). During this work he found that dilution of the rat skin extract greatly increased the specific activity of the enzyme in the extract. This finding led him to search for a reversible inhibitor(s) in the extract. During the course of that work Järvinen developed the papain-Sepharose affinity purification method for the inhibitors, which, with minor modifications, is still used all over the world (9). Two distinct inhibitors, with molecular weights of about 74000 (I_1), and 13000 (I_2) were purified and characterized. In 1976 the low-molecular inhibitor was also demonstrated in the human skin (10).

Already in 1976 we had a specific antibody of rat I_2 which I began to use in pathological anatomy in rat tissues (11). The purification, characterization and preparation of the antibody of the small molecular inhibitor from human epidermis was published in 1978 (12), and the purification of large molecular inhibitor (I_1) from human serum in 1979 (13). Later I_1 was shown to be the kininogen (14, 15).

We immediately started an immunological and physicochemical screening of the inhibitors in human and

rat tissues. These screening studies revealed that there are two different small-molecular inhibitors in the lymphatic tissue (16, 17). They had different isoelectric pHs, and the more acidic inhibitor, which was identical to that in epidermis (I_2), was called Acid Cysteine Proteinase Inhibitor ACPI (later named Cystatin A) and that with neutral isoelectric pH Neutral Cysteine Proteinase Inhibitor NCPI (later named Cystatin B) (18). Very soon it was shown the cleary different topology of these inhibitors in various tissues, e.g. in blood cells (18).

In the review article Turk *et al.* (1) the history of cysteine proteinase inhibitors starts from the chicken egg white inhibitor purified by Fossum and Whitaker in 1968 (19) and the first intracellular inhibitor from the work of Kopitar *et al* 1978 (2). The absence of the basic works of Udaka and Hayashi (1965), Järvinen (1975) and others can seemingly be explained by saying: "However, it is impossible to include most of the references due to their enormous number" (1). In my opinion, when writing a review article, it is important to bring forward a balanced picture of the development of the field. I agree that some kind of excluding of references is necessary but it must not result in the hiding of innovative key articles, lack of which could mislead the reader.

Further, the authors of the review write: "The first intracellular protein inhibitor of papain, cathepsin B and H was isolated and partially characterized from pig leucocytes and spleen" (1). The study of Kopitar (1978) contains valuable and independent information, but based on my previous discussion it cannot be the first innovative publication on intracellular cysteine proteinase inhibitors. The writers of the review (1) move forward the time invention of the mammalian inhibitors by almost 20 years. The early studies by Udaka and Hayashi (1965) from Japan and Järvinen (1975) from Finland are distinct innovations on which a great number of the later studies have been founded or at least got their basic ideas from. The information in those papers is still in most respects concrete and up to date. They can still be recommended as basic reading for young scientists working with cysteine proteinase inhibitors.

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