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PHOTOTOXIC ACTIVITY AND THE POSSIBILITIES OF ITS TESTING

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SUMMARY

The photodynamically active compounds have been attracting an attention of specialists for relatively long time. The most fruitful period of their research has been probably the last decade. The structures of the photosensitizers are very different. The biological activity is based on a formation of free reactive radicals after an excitation of the molecules of the phototoxins by e.g. UV radiation. Some photosensitizers are used for treatment of various diseases in dermatology and oncology. Since the substances can occur in plants, food or cosmetics, the photodynamic activity of these compounds is necessary to be studied under various conditions. Now e.g. some photochemical reactions, cell cultures, cultures of microorganisms, suspensions of erythrocytes, and different species of animals are used to the research of the photodynamic activity of the substances.

Key words: photosensitizers, phototoxicity, phototoxicity testing

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INTRODUCTION

Phototoxicity is defined as a toxic response that is elicited after the first exposure of the organism to certain chemicals and subsequent exposure to UV radiation or visible light. This reaction depends on the dose of the radiation and the concentration of the photosensitizer in the organism. In contrast to photoallergy, the immune system does not play such important role although a participation of some mediators (1, 2) (e.g. complement, histamine and eicosanoids) may be assumed.

CHARACTERIZATION OF PHOTOSENSITIZERS

The structures of the photosensitizers are relatively different. Nevertheless, an aromatic system and/or conjugated multiple bonds are present in their molecules. The photodynamically active substances occur in the environment or take a share in the metabolism of organisms. Typical plant constituents with phototoxic properties (Fig.1) are for example derivatives of furanochromone (e.g. psoralen) (3-6) presented in species of families e.g. *Apiaceae*, *Moraceae* and *Rutaceae*, derivatives of naphthodianthrone in species of families *Hypericaceae* (e.g. hypericin) (7) and *Polygonaceae*, polyacetylenes formed mainly in the plants of families *Apiaceae*, *Araliaceae* and *Asteraceae* (e.g. α-terthienyl) (3, 8-11). Another group of photodynamically active substances are derivatives of porphyrin (e.g. chlorophyll) (3). The photosensitive potential was shown also by some vitamins (e.g. riboflavin or pyridoxine) (12-14). A lot of medicaments can cause also photosensitive reactions of the organism. Characteristic members of such drugs are some antibiotics, quinolones, derivatives of phenothiazine (e.g. chlorpromazine), sulphonamides, some anti-inflammatory drugs (e.g. derivatives of arylacetic and 2-arylpropionic acids) and many other compounds (15). The photodynamic activity can be demonstrated also by some environmental pollutants (e.g. polycyclic aromatic hydrocarbons) (16, 17). On the other hand some photosensitizers have been used for the therapy of some diseases in dermatology and oncology (18, 19). It shows that studying of photodynamically active substances becomes an important point of view for specialists of food, pharmacy, cosmetology, ecology, hygiene and many further fields.

POSSIBLE MECHANISM OF THE ACTIVITY

On absorption of the quantum of energy, the photosensitizer molecule can be excited. Further processes may be divided in processes independent of oxygen or processes dependent on oxygen (4, 5, 15, 21). After electron or hydrogen transfer the excited molecule of photosensitizer can attack directly the molecules of nucleic acids, proteins or unsaturated fatty acids. Thus some biological properties of cell structures are changed.

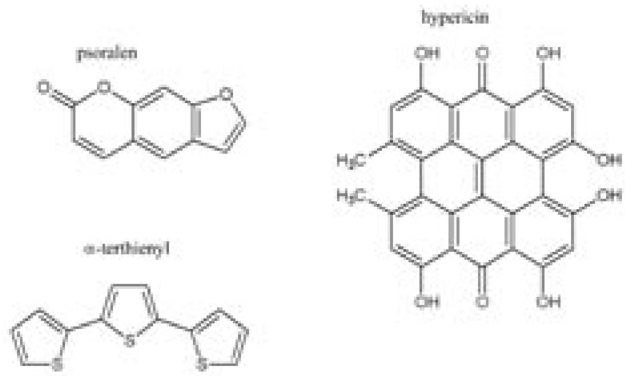

Fig. 1. *The example of some photosensitizers.*

The excitation energy of the photosensitizer can be transferred to molecular oxygen that is exited to a singlet state. This singlet molecular oxygen is highly reactive.

Further possibility is formation of the reactive superoxide anion radical after direct or indirect electron transfer between the molecules of photosensitizer and oxygen. This superoxide anion radical can undergo Fenton reaction which produces a strong cytotoxic hydroxyl radical.

POSSIBLE METHODS OF THE PHOTOTOXICITY TESTING

First, the skin of human volunteers or experimental animals was as a rule used for phototoxicity testing but the development of some ethical problems has brought, mainly in the last decade, a lot of endeavours to develop new screening methods. The possible approach consists often of several steps (20, 21). The first step should be determination of UV/VIS absorption spectrum of a potential photosensitizer. Further important steps are the studies of various photochemical reactions which the photodynamically active molecule might undergo. The photodegradation products can cause allergic reactions. Simulations of the photosensitizer behaviour at presence of different biomolecules afford also some interesting information. Peroxidation or photoadducts of unsaturated fatty acids (22, 23) or a product of their oxidative degradation – malonedialdehyde (24), photoreaction and photooxidation products of cholesterol (13, 25), histidine (20, 26) or deoxyguanosine (27) and the products of protein photobinding (20, 28) can serve as examples of such reactions.

The above-mentioned tests can be combined with other methods using cultures of various microorganisms (29) or cells. A very prevalent method is 3T3 NRU Phototoxicity Test (30, 31). There is also assay using a human skin model (32). Another method is a determination of photohaemolysis or a production of methaemoglobin in a suspension of erythrocytes (12, 33).

The cultures of various microorganisms have been already widely applied for relatively long time for the phototoxicity research. They took part in the biological activity studies of e.g. thiophene polyacetylenes (10).

The all mentioned tests work only with relatively simplified models. Therefore methods, which focus on different invertebral animals e.g. crustacean genera *Daphnia* (17), *Artemia* (6, 16, 17) and others invertebrates (11, 17), appear very promising. Although the alternative screening phototoxicity tests are developed very fast, their meaning is still mainly in the field of preliminary methods. A lot of substances manifesting their phototoxic activity in vitro may show no such effect in vivo or inversely. This phenomenon is caused by physico-chemical properties of the substance and also by the character of the live organism. It seems from this point of view that importance of vertebral animals cannot be substituted. There are e.g. a standard protocol for phototoxicity testing which employs guinea pigs (34) or a method using mice (35).

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