CYCLODEXTRIN DIMERS USED TO PREVENT SIDE EFFECTS OF PHOTOCHEMOTHERAPY AND GENERAL TUMOR CHEMOTHERAPY

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ABSTRACT

Recent developments of polyphasic tumor detection and tumor therapy including applications of dimeric cyclodextrin inclusion complexes of high affinity for Photodynamic Tumor Therapy (PTT) porphyrinoids are discussed in the light of future applications of similar methodology to general tumor chemotherapy. These approaches hopefully will end the martyrium of patients undergoing tumor chemotherapy today.

1. INTRODUCTION

Up to now, there are only two approaches seriously to be discussed for a tumor selective targeting of substances either to detect or to destroy tumors.

One is devoted to radiological detection of tumors using biotinylated monoclonal antibodies and a biotinylated chelating agent to transport γ-radiating isotopes. The connecting member is the egg protein avidin or the bacterial streptavidin which bind with high affinity (10^{15} l/mole) to biotin. After injection of the monoclonal antibody into the blood stream and its fixation to the tumor considerable amounts of antibody molecules remain in the circulation. These are chased off by an injection of avidin or streptavidin which label by the same time the tumor bound antibody. Finally the radioisotope carrying chelate is injected which binds exclusively to the tumor. By this method *Fazio a. Paganelli*[1] were able to localize just liver metastases which are normally hidden by a heavily scintillating background in this organ.

The second method uses an ingenious biotechnological construct consisting of the active center of a mouse monoclonal antibody directed against the cell bound tumor marker CEA (*C*arcino-*E*mbryonic *A*ntigen), F_c-parts of human immunoglobulin, and a normally only intracellularly displayed enzyme, β-glucuronidase, to label adenocarcinoma and its

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metastases. After injection, the construct labels the tumor and is slowly washed out from the circulation. Then the natural detoxification product of the antibiotic adriamycin, adriamycin glucuronide, is injected which is split and therefore retoxified exclusively at the tumor site. By this method *Bosslet and his coworkers*[2] were able to kill completely adenocarcinomas without the dangerous side effects of adriamycin to the heart which normally prevents a longer and high-dose application of this drug. This approach is now in phase II clinical investigations.

When we intend to prevent the side effects of tumor chemotherapy which lead to a martyrium of the patients we have to learn from these both successful approaches. The first we can learn is that an antibody recognizing a tumor site must be humanized or labelled only with a minimum structure like biotin to slip through the rigorous control of the reticuloendothelial system preventing foreign molecules to reside for a longer time in the human body. The second lesson is that only drugs with an excretion signal or, broader speaking, a high hydrophilicity preventing uptake into any living cell combined with an attachment signal structure will allow for a successful specific labelling of tumors.

This in mind we tried to form inclusion complexes of porphyrinoid drugs for use as photosensitizers in Photodynamic Tumor Therapy (PTT) with a shell of dimeric or trimeric cyclodextrins in order to prevent any uptake into living cells[3]. We attached biotin with a long spacer molecule to the shell or to the porphyrinoid itself as a signal structure. And we begun first experiments to localize such drugs at tumor sites.

2. MATERIALS AND METHODS

Cyclodextrins and methyl β-cyclodextrin (1.8 saturation) were obtained from *Wacker-Chemie (Burghausen, Germany)*. β-Cyclodextrin dimers connected with alkyl spacers of different lengths .were synthesized according to *Ruebner et al.*[4] (see the accompagnying poster paper). Complex stability constants with tert.butyl phenoxy substituted porphyrinoids were determined by fluorimetry using guest exchange between p-toluidino naphthalene sulfonic acid and the respective porphyrinoids[5]. Tert.butyl phenoxy substituted porphyrinoids were kindly gifted by *Dr. M. Müller (Laboratory of Prof. Dr. E. Vogel, Organic Chemistry, University of Cologne, Germany),and by Andrea Weitemeyer (Laboratory of Prof. Dr. D. Wöhrle, Organic and Macromolecular Chemistry, University of Bremen, Germany)*. Attachment of spacered biotin followed the route of reaction of the free acid groups with carbonyl diimidazole and coupling with biotin cadaverine in dimethyl formamide. Similar products can be obtained by coupling via dicyclohexyl carbodiimide or - on NH_2 groups - with biotin-X-NHS.

Absorption spectra were obtained with a Shimadzu UV 200 instrument at 2 nm resolution, fluorescence spectra with a Shimadzu 1501 instrument at 10 nm resolution. Mass spectra were of MALDI type obtained on a LAMMA 1000 spectrometer with 2,5-dihydroxy benzoic acid as the matrix.

3. RESULTS AND DISCUSSION

γ-Cyclodextrin was sometimes used to solubilize photosensitizing porphyrinoids, like tin etiopurpurins, for injection purposes[6]. We found monomeric β-cyclodextrin and its methylated derivative much more suitable to solubilize e.g. pheophorbide a, phthalocyanines[4], or porphycenes[3]. Complex binding occurs in a two step reaction where the first step, i.e. desaggregation of dye polymers, is velocity-limiting , and so masks the real complexation process[3]. As a result we obtained very low binding constants[7] which could be corrected only by the guest exchange procedure using fluorimetry of p-toluidino naphthalene sulfonic acid[4,5]. Injection of porphyrinoids bound to monomeric cyclodextrins with binding constants in the order of $3x10^3$ [l / mol] leads to exchange with the lipoprotein fraction in blood plasma[7, 8]. In cell culture we see an enhanced uptake into tumor cells possibly due to a directing of the dye molecule perpendicularly to the cell membrane with its lipophilic end leading to a substantial incorporation of the drug into lipophilic parts of the membrane and, consequently, to a considerably better uptake into the cell. An example shows fig. 1 for the uptake of glutaryl amido tetra propyl porphycene (GlamTTPn).

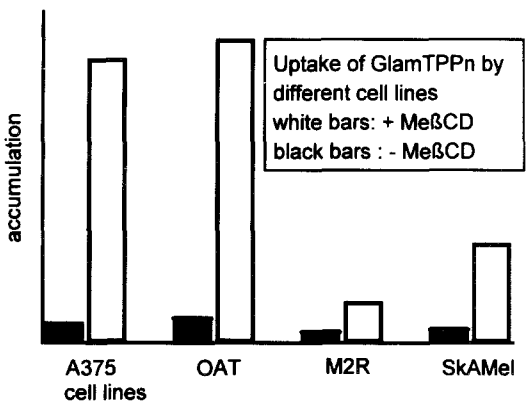


Fig. 1 Uptake of GlamTTPn into different cell lines with and without prior complexation to methyl β-cyclodextrin.

For high affinity inclusion only dimeric taylored β-cyclodextrins are suitable. The prer-equisite are modified peripheries of the porphyrinoids since the normally less voluminous peripheral side-groups of native porphyrins cannot dive deeply enough into the lipophilic cavity of the cyclodextrin torus. *Breslow a. Chung*[9] have synthesized model compounds

containing voluminous tert.butyl benzoic acid groups on both ends allowing for binding constants of about 10^{10}[l/mol] with sterically hindered cyclodextrin dimers.

The substitution of phthalocyanines, *meso*-tetraphenyl porphyrins, and pheophorbides with tert.butyl phenoxy or tert.butyl benzoic acid substituent led to affinity constants of 10^7 l/mol upon inclusion into β-cyclodextrin dimers with spacers of 11-13 C-atoms lengths[10,11]. Stability of these inclusion complexes against the lipoprotein system of blood plasma has been proved by gel permeation chromatography and absorption spectroscopy[12]. Stability against transfer of the guests into cell membranes are under investigation. The drugs synthesized up to now are model compounds which have to be replaced further by effective photosensitizing drugs alike the one shown in fig. 2.

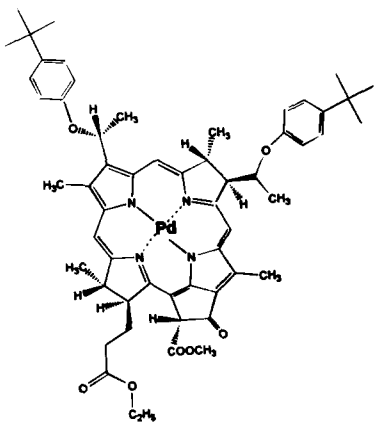


Fig. 2 Ideally substituted bacteriopheophorbide sensitizer as prepared now from bacteriochlorophyll g of a recently discovered *Heliobacterium*[13]

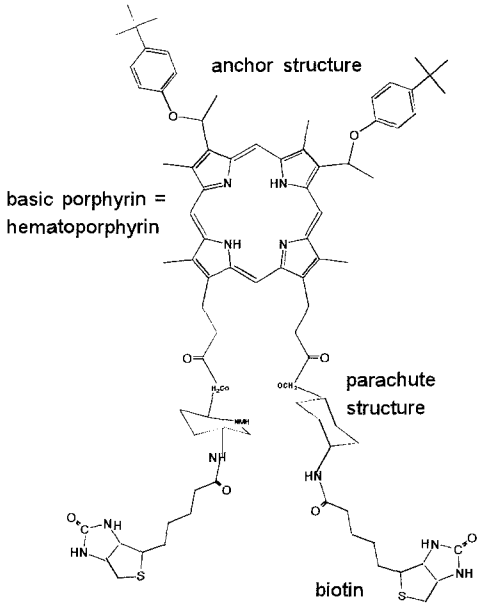
Such photosensitizers are easily biotransformed into inert splitting products which overcome the longtermed photosensitation in patients treated by PDT[14].

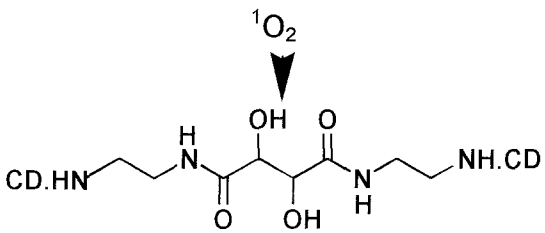
To bind such sensitizers exclusively onto tumor cells a polyphasic system based on biotin-avidin interaction has been formulated which is now under investigation in model animal experiments[15]. Such systems were successfully introduced into radiological detection of tumors[1] and seem to work as well with biotinylated photosensitizers included into cyclodextrin dimers so long as the spacer structures involved are not too extended[16]. An example of such a construct is shown in fig.3

To set free the included drugs at the tumor site preformed breaking points inside the spacer structures were provided. For $^1O_2$-producing porphyrinoids such a structure can be a simple tartric acid inset sensitive to activated oxygen species (fig.4). Upon opening the

bridge the affinity constant drops by 3-4 orders of magnitude and the drug can entry into the next-neighboured cell. Several other types of laser sensitive bridges were conceptually possible and are just synthetized in part[17] for other purposes.

(3)





(4)

Fig. 3 Biotinylated di-tert.butyl phenoxy hematoporphyrin as included into a taylored β-cyclodextrin dimer

Fig.4 A $^1O_2$-sensitive breaking point structure allows for setting free the guest molecule.

We should realize that photodynamic therapy is only a very small cutting out of the manifold of methods in general chemotherapy with its far-reaching side effects. The long-term neurotoxic and genotoxic events have to be avoided in future. We have learned a lot from photodynamic therapy drugs to transfer this knowledge now to general chemotherapeutic drugs which work up to now according to the rule:"We poison the whole patient and hope that the tumor will die a little bit earlier as its carrier". There are several highly poisonous drugs which cannot be used for tumor treatment because of its high general toxicity. Overviewing the chemical structures of such drugs there are a manifold which presumably can be included into inert carriers alike those developed for PDT drugs, i.e. cyclodextrin dimers. One of the most interesting of these is the 25 years old microtubule hyperstabilizing drug, paclitaxel or taxol. This drug can be given to patients resistant to all other chemotherapeutic regimes due to its uncommon mechanism of action. This drug with its 3 phenolic groups is a model case of possible inclusion into a taylored cyclodextrin trimer which is now under investigation in our laboratory. First experiments show a consistent binding into cyclodextrin monomers.

4. CONCLUSION

Dimeric and trimeric cyclodextrins will be useful tools to detoxify and to direct anti-tumor drugs to its target. These coming developments should be of interest not only for medical doctors but for the pharmaceutical industry. Experimental designs are not easily projected into industrial realization. So far, these should be accompagnied in an early stage by industrial partnership.

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