DRUG GLYCOSIDATION-A RECENT APPROACH IN DRUG TARGETING

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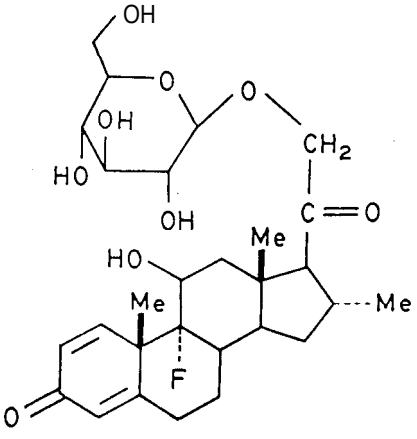
An important role of the medicinal chemist is to improve upon existing drugs by increasing their potency and duration of action and by decreasing their toxic side effects. A drug molecule with its optimal structural features and physico-chemical properties to eliciting the desired therapeutic response may not necessarily possess the best molecular form and properties to reach the target site effectively. An insufficient delivery of the drug molecule to its site of action often results in undersirable side effects. Of recent, there has been a great awareness in the concept of drug targeting the objective of which is to deliver a drug to a specific site (organ, tissue or cell) in the body to maximise therapeutic effect and reduce toxicity. There has been development of different approaches to site-specific drug delivery such as implantable pumps (Shaw, 1980), adhesive patches impregnated with drugs (Shaw, 1980), vesicle-enclosed drugs (Tundo et *a/,* 1982; Regen et *al,* 1982) and drug carriers (Cregoriadis, 1979). Prodrugs have also been used in targeted drug delivery (Bundgaard, 1983; Sinkula and Yalkowsky, 1975; Stella, 1975; Stella and Himmelstein, 1980; Stella et *al,* 1980). A prodrug is a latent form of an active drug with certain physicochemical properties that allows it to reach the target organ or tissue. Once there, the active drug is formea chemically or enzymatically in situ. Drug glycosidation is one of the most recent approaches emerging in the area of drug targeting, and has been successfully employed in the synthesis of certain site-specific drugs. The present article highlight\ some of the recent advances made in the area of drug glycosidation, the role of sugar as a carrier molecule and the problems of glycoside synthesis.

Clycosides are found in nature and in a way offer an example of naturally occurring prodrug. Glycosides such as amygdalin and cycasin are hydrolyzed by gut microorganisms to mandelonitrile and methylazoxymethanol, respectively (Scheline, 19731. The naturally occurring cardiac glycoside lanatoside C is hydrolyzed to bioactive digoxin in the gastrointestinal tract hy both bacterial and chemical action (Beermann, 1972). An organic molecule containing an appropriate group can, however, be converted to its glycoside derivative synthetically by well established methods (Garegg et al, 1985; Wulfi and Rohle, 1974). Such derivatisation which results in change in the physicochemical properties of the original molecule has earlier been used in the enhancement of absorption, inc-reased aqueous solubility, facilitate transport and as protective groups. β-Clucosideof menthol represents an exampleofa nonirritating water-soluble derivative of menthol. Xanthotoxol, an antiadrenergic and CNS depressant hydroxyfuranocoumarin, has been converted into its D-glucoside derivative to improve the ease of formulation (lain et a/, 1985). More recently the concept of drugglycosidation i.e. conversion of a drug molecule to its glycoside by synthetic methods has been applied rationally in the design of site-specific drug delivery systems. The following examples will illustrate the successful utility of this approach.

Site-specific Anti-inflammatory Agents

The first application of the concept of drug glycosidation can be traced back to 1964 when synthesis of 11 β, 17-dihydroxy-3, 20-dione- 1,4-pregnadien-21 -yl 2-acetamido -2-deoxy-β-D-glucopyranoside was achieved to release the active drug in the synovial fluid of rheumatoid arthritis victims after cleavage by βN-acetylglucosaminidase (Hirschmann et *a/,* 1964). A striking increase in the level of the enzyme wasalready reported for the synovial fluid ofarthritis patients. After a gap of twenty years the approac h was again utilized in the colon-specific delivery oi some anti-inflammatory steroidal drugs (Friend and Chang, 1984; Friend and Chang, *1985).* The well documented importance of the ability of the gut microflora to hydrolyze glycosides

was effectively applied by Friend and Chang (1984; 1985) when they synthesised the 21-yl β-D-glucosides and galactosides of dexamethasone, prednisolone, hydrocortisone, and fludrocortisone and the 21-yl β-D-cellobioside of prednisolone by a modified Koenigs-Knorr reaction. Glycosides of these drugs are larger and usually more hydrophilic than the drugs themselves. As such the penetration of these glycosides across biological membranes is reduced. These prodrugs on oral administration are not cleaved by the digestive enzymes of the upper ,intestine and they pass unabsorbed into the colon, where bacterial glycosidases can hydrolyze them. This results in the release of bioactive lipophilic drug to be absorbed by the colonic mucosa. This remarkable approach may be useful in the treatment of inflammatory bowel conditions. The *in* vitro experiments carried out revealed that the delivery of dexamethasone via its glucoside (1) was more specific than that of prednisolone glucoside. The concept offers a considerable scope for the colon-specific delivery of other drugs such as antibiotics with fewer systemic side effects. Likewise, the fact that colon also has azo-reductase activity of its microflora can also be effectively utilized to deliver active pharmacon to the colon by designing azo bonds containing prodrugs.



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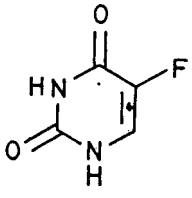
Site-specific Antineoplastic Agents

Another promising area where the approach ofdrugglycosidation is being effectively utilised is in the design of site-specific antineoplastic agents. Elevated β-glucuronidase activity in malignant tumour was first reported by Fishman and Anlyan (1947). Subsequently many reports confirmed this fact and today we know that there exists higher levels of β-glucuronidase in human cancer tissues relative to normal tissue (Lewy and Conchie, ***1966).*** It was shown that mycophenolic acid, a compound which inhibits mecca lymphosarcoma and CA-755 mammary carcinoma, is converted into the glucuronide as theonly metabolite. Further, it was shown that, in general, the tumours most responsive to mycophenolic acid are those in which β-glucuronidase activity is highest, while it is the lowest in nonresponsive tumours. It was demonstrated that the N,N'-bis-(2-chloroethyl) aniline is oxidized in vivoto the corresponding 4-hydroxy derivative, which is then converted into its glucuronide (Connors ***et al,*** 1973). This aromatic nitrogen mustard is effective against mouse ADJ/PCS plasma cell tumour which exhibits particularly high levels of β-glucuronidase activity. Further the activity of β-glucuronidase is enhanced cells,become more acidic, since the pH-velocity maximum of the enzyme is between 4 and 5. It is already known that the acidity of cancer cells is more than the normal ones and it gets increased specifically by glucose. Taken together, these observations suggest that glucuronides (glycosides) of known anticancer compounds can selectively deliver these drugs to cancer tissue. This approach has successfully been applied by several workers.

5-Fluorouracil (2) is one of the most prominent antitumour agents used in the treatment of certain solid tumours (Reynolds and Prasad, 1982). It gives rise to a number of side effects. A number of its derivatives have been synthesised and their anticancer activities have been investigated. Keneko ***et al (1977)*** reported the first synthesis of glucuro-



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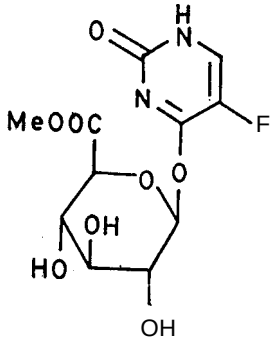
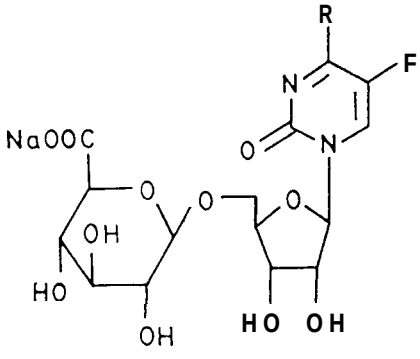


(3)

nidederivativesof 5-fluorouracil. Mythyl 1-(5-fluoro-1 H-2-oxo-pyrimidine- 1 -yl)-βD-glucopyranuronate (3) was prepared by a condensation reaction of the silver salt of 2-benzoyloxy-5fluoro-4-pyrimidone with 1 -bromo-1-deoxy-2,3,4-tri*O*-acetyl-α-D-glucopyranuronate and subsequent careful removal of the protective groups. The corresponding β-D-glucopyranuronamide was also prepared. The glycosidic derivative (3) has shown a high degree of tumour-specific activity in mice with transplantable solid tumours than the parent drug. Earlier, synthesis of unprotected 2,4-bis-O-glucoside of uracil and thymine has been reported (Rogers ***et al,*** 1969).

Recently, Watanabe ***et al*** (1981) employed the glycosidation approach in a more rational way to prepare the masked precursors of anticancer nucleosides. It is reported

that the corresponding ribonucleoside and deoxyribonucleoside of 5-flurouracil are also active. These compounds are converted in vivo into 5-fluorouracil deoxyribonucleoside-5'-phosphate, which then interferes with thymidilate synthetase, or are metabolised to 5 fluorouracil ribonucleoside-5'-phosphate which eventually is incorporated into RNA, producing some abnormally functioning RNA species. Regardless ofwhich mechanism operates, metabolic conversion of these drugs into 5'-nucleotides by cellular enzymes is a pre-requisite for a chemotherapeutic effect. One would thus expect ,that 5'-glucuronides of such nucleosides would be relatively inactive.Enzymatic hydrolysis of the glucuronide moiety from such nucleoside glucuronides which could enable subsequent entry into nucleotide metabolic pathways may be expected to occur more readily in tumour tissue rich in β-glucuronidase resulting in selective toxicity. The nucleoside 5'-glucuronides, like other glucuronides would be resistant to chemical hydrolysis under physiological conditions. Further, such nucleoside glucuronides would probably not serve as substrates for nucleoside phosphorylasedue

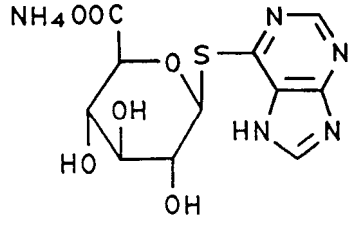


(4) R = O H

(5) R=NH2

to high degree of structural specificity which is the requirement of such enzymes. Thus, the only metabolic fate of the glucuronides would be cleavage by β-glucuronidase liberating biologically active nucleosides. On this basis 5'-*O*-β-D-glucuronides (4,5) (isolated as sodium salts) of anticancer nucleosides, 5-fluorouridine and 5-fluorocytidine were prepared (Watanabe, etal, 1981). These glucuronides we're substrates of both bacterial and bovine β-glucuronidase. Both were, as expected, much less cytotoxic relative to their parent nucleosides against P815 cells, the LD_{50} values for the glucuronides were both approximately 6μg/ml, whereas those for the parent nucleosides were found to be in the 0.004-0.006 μg/ml range.

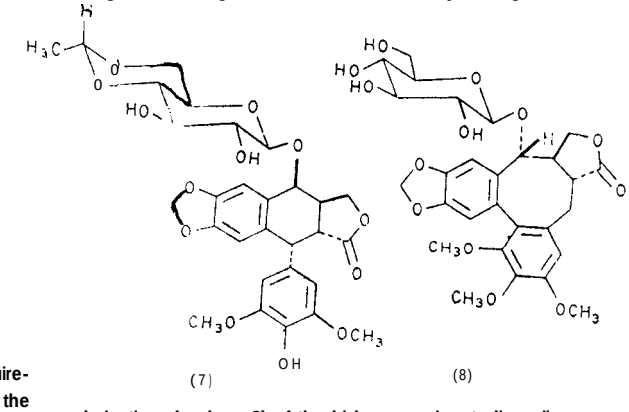
A glucuronide of 6-mercaptopurine, ammonium 7H-purin-6-yl- 1 -thio-β-D-glucopyranosiduronate (6), synthesised by Parker and Fedor (1982) has been found to be as good substrateof β-glucuronidase. Theglucuronidecaused a 22% decrease in the growth of L1210 cells while not affecting the growth of Chinese hamster lung fibrobiasts from a nontumour line. The corresponding amide, 7H-purin-6-yl-1 -thio-β-D-glucopyranosiduronamide was also found to be a substrate, albeit a poor one, for a β-glucuronidase. It is believed that the substrate behaviour of



(6)

the amide is not due to a nonspecific protein effect and its hydrolysis occurs in the enzyme active site. It had, however, no effect on the growth of either cell type.

Modifications of active plant principles have, in several cases, provided clinically useful agents. Among the premier examples of such successful modifications are the glucosidic derivatives of 4'-demethyl-epi-podophyllotoxin VM-26and VP-I 6-213 (7), which are clinically useful anticancer drugs. Continuing in this direction, recently, the glucosidic



(7) (8)

derivatives (such as 8) of the bisbenzocyclooctadiene lignan lactone steganol analogous to VP-I 6-213 have been prepared (Hicks and Sneden, 1985; Houlbert et al, 1985).

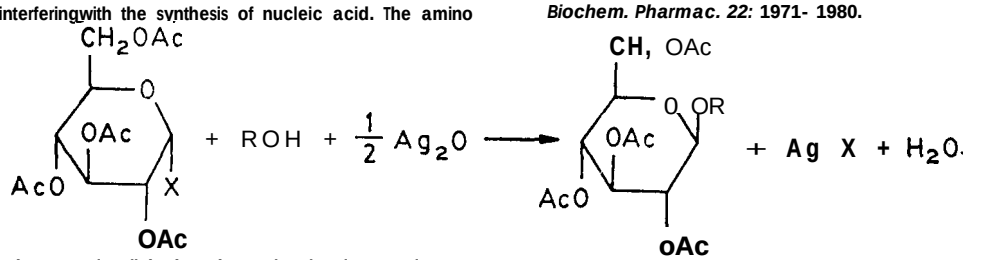
Glycosides as Drug Carriers

Recently the application of synthetic alkyl glycoside vesicles as drug carriers has been reported (Kiwada et al, 1985). It was observed that alkyl glycosides formed lamellar vesicles like phosphatidylcholine vesicles (liposomes). Various alkyl glycosides were synthesized and vesicles were prepared with these glycosides. The encapsulation capacity of the vesicles was examined in relation to alkyl chain length, sugar moiety, and lipid composition. The glucosides of myristyl, cetyl, and stearyl alcohols formed vesicles, but

those of lauryl, decyl and octyl alcohols did not. The vesicles of glucoside, galactoside, and mannoside showed fairly good encapsulation capacity but those of lactoside showed low capacity. The alkyl glycoside vesicles show longer lives on stage in an ampoule at 20°C than phosphatidylcholine vesicles. These have also shown rapid release of about 40% of the aqueous contents, but after that, they showed outstanding.stability for 48 hours in plasma at 37°C. In comparison phosphatidylcholine vesicles showed rapid release of only about 30%, and disintegrated gradually and showed low encapsulation capacity (about 20%) after 48 hours.

Significance of Sugars in Glycosides

Sugars are the natural products formed by enzymatic manipulations in bio-systems and, as such, are inherently more likely to be accepted by the human body. The significance of sugars in naturally occurring glycosides has long been recognised. The cardiac glycosides areone of the most important and widely used groups of drugs in clinical medicine. The biological activity of these molecules is thought to arise as a result of a specific interaction between a receptor and the aglycone portion of the molecule. The presence of he sugars retards detoxification effectively and confers on the molecule certain physical properties important for uptake and distribution. There are some evidences that the action of cardiac glycosides depends upon active transport into the myocardium. It is reported that the potency of the steroidal aglycone is greatest when the β-hydroxy group at C-3 is combined as a glycoside. In case of anthraquinone glycosides which are employed as purgatives, the sugar serves to transport the aglycone to the site of action in the large intestine. Without the sugar moieties free anthraquinone exhibit little therapeutic activity. Daunomycin, an antineoplastic antibiotic, acts by binding tightly with DNA and interferingwith the synthesis of nucleic acid. The amino



sugar daunosamine linked to the molecule plays an important role in this binding (Hollstein, 1979).

In nature the most common sugars occurring as glycosides are D-glucose, D-glucuronic acid, D-xylose, L-arabinose, D-galactose, D-galacturonic acid, L-fucose and L-rhamnose. In glycosides where oligosaccharides form the sugar component hexoses occur 'oh the inside' (i.e. linked directly onto the aglycone) and pentoses and methyl pentoses 'on the outside'. Oligosaccharide moieties containing more than two sugars often appear to be branched (Reichstein, 1962).

Preparation of 0-Glycosides

The mechanisms and problems of O-glycoside synthesis have been excellently reviewed (Wulff and Rohle, 1974). The glycosides are prepared by modified Koenigs-Knorr reaction; the original form of this method was developed in 1901. Acylated 1 -halo sugar which is obtained as thermodynamically more stable a-form (halogen is axial) is used as the starting material. Practically only chloro-and bromo-derivatives are used in glycoside synthesis. The halogen atom of these acylated 1 -halo sugars is very active and its reaction with an alcohol in the presence of an insoluble silver salt such as silver oxide or silver carbonate gives the β-glycoside. The water formed in the reaction often reduces the yield. Various modifications have been made to remove the water from the reaction mixture, such as addition of an inorganic dessicant in the mixture or distilling off the water as an azeotrope with benzene. It is reported that the use of silver salt of a hydroxy carboxylic acid gives better results and the reaction can be carried out even at-10° and therefore suitable for highly sensitive alcohols. Deacylation of the acylated glycoside is carried out by a base catalyzed hydrolysis.

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