

SESSION 3

THERAPY OF HERPES SIMPLEX ENCEPHALITIS

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Molecular pharmacology of the pyrimidine analogues

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PYRIMIDINE analogues fall into five main groups: (1) the aza compounds, where a nitrogen replaces the carbon, either in the 5 or 6 position; (2) the halogenated compounds, where the halogen (fluorine, bromine or iodine) is replacing the methyl of thymidine in the 5 position; (3) compounds in which the sugar moiety, the ribose or deoxyribose, is changed to something else such as arabinose, for example; (4) in a broad sense one might call analogues those compounds which somewhere in the structure contain a radioactive atom, such as tritium, either in the methyl group, or somewhere in the ring; (5) one may consider a number of loosely related compounds which one way or another act as analogues within synthetic pathways.

With pyrimidine synthesis, the ring is synthesized first, and is then coupled to a sugar, unlike purines, which are synthesized from the beginning with the sugar part attached. Pyrimidine ring synthesis starts off with carbamyl-phosphate and aspartate, forming carbamylaspartate, which then forms dihydroorotate by cyclization. This step can be inhibited by aza compounds, azaracil or azauridine. The cyclized dihydroorotate is converted to orotate which then couples with sugar and phosphate (making orotidine-5-phosphate) using the so-called PRPP reaction (phosphoribosyl pyrophosphate with appropriate enzyme). This is strongly inhibited by fluoro-orotate, azaotic acid or uracil methylsulphone. The orotidine-5-phosphate is then decarboxylated to uridylylate. This decarboxylation is inhibited by the aza compounds. Therefore, the neosynthesis of uracil or uridine is thereby inhibited, and the cell accumulates orotidine, and turns into an unbalanced growth state in which it dies, unless the block is lifted with preformed uridine. The preformed uridine, although it does not lift the block in the sense that the orotidine is still accumulating in the cell, still allows nucleic acid synthesis, however, because it can be utilized.

It is worth remembering that in the case of pyrimidines (unlike purines) the naked ring form can be very poorly utilized by mammalian cells. In the case

of purines, adenine is far better utilized by mammalian cells than, for example, adenosine or adenylic acid. In the case of pyrimidines, uracil, cytosine, or thymine, are very poorly utilized by mammalian cells, and it is in combination with their sugar (uridine, thymidine, cytidine) that the cells are able and effective in taking them up.

There is a whole chain of interconversions to be considered, since the reduction of the ribosyl to the deoxyribosyl form is the main source of deoxyribose in mammalian tissues. This can be effectively inhibited by any excess deoxyribosides (by simple feedback mechanism); among them of course, the halogenated deoxyribosides, which are just effective, or slightly more effective, than an excess of normal deoxyribosides. The same reaction is also inhibited by a compound called hydroxyurea, which blocks the ribosyl-deoxyribosyl transformation. In this case the hydroxyurea 'block' can be overcome by providing appropriate deoxyribosides. Because of the above-mentioned feedback inhibition by excess deoxyribosides, however, the block which one can lift from hydroxyurea has to be lifted with carefully dosed deoxyribosides.

Cytidine can be effectively transformed to deoxycytidine which, in turn, can be deaminated, then methylated, thus forming thymidine. Uridine can also be transformed into the deoxycompound, and methylated into thymidine, but somewhat less efficiently than cytidine. Hence, uridine is, by and large, a better 'specific' precursor of RNA when it comes to labelling studies than cytidine which is more ready to be transformed into deoxycytidine, and therefore incorporated into DNA. The amino group for cytosine neosynthesis usually comes from some glutamate compound, and this pathway can be effectively inhibited by appropriate analogue, diazo oxonorleucine (DON), but interestingly enough, once the pyrimidine ring has been formed and coupled to a sugar, forming say uridine, the amination of uridine into cytidine does not require the nitrogen from glutamate and DON does not inhibit it. On the contrary, it pushes the reaction because it

prevents the preformation or neosynthesis of the ring state.

The cytosine deoxyriboside deaminase, which is required to transform the compound into thymine, is inhibited by the same TTP feedback, i.e. deoxyriboside triphosphate excess feedback, and even more effectively by the halogenated compounds. Finally, it should be recalled that the methyl group of thymine requires the methyl transfer (folate) pathway.

As to the effects of these five groups of compounds; the aza compounds, mentioned already, interfere with various pathways of the pyrimidine ring synthesis and its glycosylation, and they are quite effective in inhibiting neosynthesis, but not very effective in inhibiting interconversions of ribosides to deoxyribosides. Hence, they are, by and large, not very toxic compounds because some preformed nucleotides always occur in the body from breakdown of other cell types which can be therefore reutilized instead. The one compound in this group which is somewhat more effective than the rest is azathymidine (with a nitrogen in the 6 position). This acts similarly to any of the other thymidine analogues, such as iododeoxyuridine, but is less effective and less toxic. Azauridine has a moderate effect on RNA synthesis by impairing the synthetic pathways, but it can be compensated by the breakdown products, uridine or cytidine, quite effectively. Nevertheless, in large enough doses, azauridine has toxic effects manifest in CNS disturbances, eventually resulting in convulsions. Azacytidine, which has a longer plasma clearance, since it is less easily broken down, has some cytotoxic effect in certain experimental mouse leukaemias. None of these compounds has, however, fulfilled its initial chemotherapeutic promise.

If we leave for the moment the halogenated compounds and take the third group of analogues, i.e. where the sugar moiety is changed, the most notable compound is cytosine arabinoside. This is an interesting compound in so far as it is very effective against a number of experimental mouse tumours, but remarkably ineffective in rats. It is used in human malignancies, in treatment of acute leukaemia, particularly acute myeloid leukaemia. It is a fairly toxic compound, and at the moment the dosage regimen is of the order of 3 mg/kg for 3–5 days, and then some interruption of treatment is required because of the cytotoxic, depressant effects. Like azauridine, it does not inhibit the uridine incorporation into RNA, and it does not block the preformed uridine incorporation, but it effects DNA synthesis quite remarkably. In that, of course, its effect can be overcome by providing competing concentration of cytosine deoxyriboside, which then lifts the block and prevents, or stops, the further

toxic symptoms from developing. Resistance against the compound can develop, because for its transport into the cell a specific kinase is required, the Ara-C kinase, and kinase-less mutants can develop. This kinase is different from thymidine kinase, therefore cells which lack the thymidine kinase will still be sensitive to Ara-C and vice versa.

Another form of the sugar analogue is one where the sugar moiety is not the normal for that particular compound, and this is thymine riboside. Thymine normally occurs in the deoxyribose form but we know that in some types of RNA some traces of thymine ribosides are normal constituents. Nevertheless, an excess thymine riboside, as opposed to deoxyriboside, is cytotoxic, although not much more than azathymidine, i.e. it is not a very efficient analogue. Its effect can be overcome by thymine deoxyriboside.

The fourth group is the radioactive group of 'analogues', and in this case the most commonly used radioactive atom is tritium, which can be introduced anywhere in the ring, or, in the case of thymine, into the methyl group. This gives, of course, a very specific possibility of introducing radioactivity into the cell, and since the radioactive emission from tritium is an extremely soft β -particle with very limited penetration, over 90% of the radiation which is emitted within a cell nucleus will be absorbed within that particular cell nucleus. The total dose along the path of one single tritium β -particle may come to about 180 rad and therefore there is a very good chance that cells in which a number of such disintegrations occur will be sterilized by this intranuclear radiation. Therefore, tritiated thymidine is a highly effective DNA 'analogue', in the sense that it will pick up those cells, which are at that time in DNA synthesis, and will kill them quite effectively. The method has been used with certain experimental tumours, and is called the thymidine 'suicide' method (this is a misnomer of course, it would be more proper to call it thymidine 'murder').

The fifth group is a number of miscellaneous compounds and I mentioned already one such compound, hydroxyurea, which blocks the conversion of the ribosides to the deoxyriboside forms, but there are one or two compounds worth mentioning. One is 5-methyl amino-2-deoxyuridine, which is quite specific for herpes virus, but not for other DNA viruses. Otherwise it is a reasonably cytotoxic compound. Similarly, 6-uracil methylsulphone, which inhibits the PRPP reaction, is also quite effective in inhibiting herpes virus formation, and in this case it blocks the enzyme quite remarkably, so much that the effect cannot be reversed by excess thymidine.

Now, returning to the second group of compounds, that of the halogenated pyrimidines. The fluorinated

compounds are interesting in so far as on the face of it they would be perfect as thymidine analogues, because the size of the halogen atom is more or less similar to the size of the methyl group. So structurally, and as far as steric configuration is concerned, they are reasonably similar. Nevertheless, fluorouridine or fluorodeoxyuridine (FUdR), both of which can effectively get into the RNA of some viruses and produce certain mutations there, cannot get into DNA of mammalian cells. Fluorodeoxyuridine has, like all the other deoxy compounds, affinity for the kinases and synthesases and, therefore, can block DNA synthesis quite effectively, but the compound itself does not get built into DNA. Therefore, what happens is that cells which happen to be in the process of DNA synthesis at that time will be stopped. Since the DNA synthesis period in a cell does not involve DNA synthesis only, but other synthetic processes, interruption of the DNA synthesis results in an unbalanced state, which is eventually lethal for the cells. Therefore, cells inhibited by fluorodeoxyuridine in the DNA synthesis usually die. Cells which have not yet entered DNA synthesis (i.e. which are in the G₁ part of the cycle) will be prevented from entering into the S period, and there will be an accumulation of cells at the beginning of the S period. A similar block is produced by hydroxyurea and, of course, a similar block would be produced by any of the folic antagonists. So we have four ways of killing cells specifically in the S period, including radioactive thymidine. When such 'S period' blocks are lifted, the cells are almost starved for deoxyribosides and therefore an increased uptake of, for example, tritiated thymidine or halogenated deoxyribosides can be achieved; i.e. their incorporation can be potentiated with pre-treatment with FUdR.

In spite of the fact that FUdR does not get built into DNA, for its effect the kinase is necessary and those cells which are lacking thymidine kinase cannot react to fluorodeoxyuridine either.

The other two halogenated compounds, bromo- and iododeoxyuridine, (BUdR and IUdR) can be readily incorporated into mammalian DNA, but not so the ribosides. This is interesting because cytosine riboside, or uridine riboside, can be quite easily transformed into deoxyriboside and incorporated into DNA, but apparently the halogenated ribosides, although incorporated into RNA, cannot be transformed into deoxyribosides.

Iododeoxyuridine is the most effective of the halogenated analogues (more effective in cytotoxic terms than bromodeoxyuridine) and it is a specific DNA inhibitor, it does not inhibit the synthesis of the RNA pyrimidines. Iodocytosine riboside (ICydoR) is essentially an effective precursor of iododeoxyuridine because that is what it is decomposed to, but

at a slow rate, so whenever one wishes for a 'depot' effect of IUdR, administration of ICydoR might be a good way of producing it, although by and large, lower plasma levels will be achieved. Both these compounds, when incorporated into mammalian and virus DNA, will produce certain disorders, not only because of configurational changes in the DNA (because the size of the 'methyl group' has now been changed by replacing it with iodine or bromine groups) but because they are producing a permanent kind of miscoding. If the incorporated 'abnormal' base only matters, then dilution by subsequent cycles of DNA synthesis would soon render it ineffective. However, the halogenated thymidine analogues produce a miscoding. Thymidine normally pairs with adenine, and very rarely with guanine, but if the methyl in the thymine is replaced with iodine or bromine, then the chance of it pairing with guanine increases by at least fifty-fold. This produces a 'permanent' miscoding, because such a guanine will pair in the 'next round' with cytosine and the original thymine-adenine base pair has been replaced by a guanine-cytosine one.

Both BUdR and IUdR inhibit virus growth, although they may allow a delayed viral DNA synthesis. Nevertheless, the infectious virus titre usually drops in these cells, even with BUdR which allows formation of non-infective virus particles while IUdR frequently prevents formation of virus particles altogether. The iodine in the DNA may prevent the formation of protein coat in most viruses; the bromine may not prevent the formation of coat, but it may prevent uncoating in certain viruses, or perhaps produce a faulty coat, or interfere with the production of some of the early enzymes. The anti-viral effect can be seen in concentrations which are lower than immediate cytotoxic effects, although they may inhibit temporarily cellular DNA synthesis. At certain concentrations, the drug (e.g. 10 µg/ml of IUdR) will permanently inhibit the proliferative capacity of certain mouse embryo cells (and, of course, in that concentration inhibits viral proliferation in these cells), but if the cells are removed from this medium and suspended in fresh medium without the analogue in it, although the inhibition of cell proliferation remains permanent, virus proliferation can start in those cells.

The toxic side-effects of these compounds is not the subject of this paper, but in talking about molecular pharmacology their role as mutagens and teratogens had to be mentioned. Also, the halogenated pyrimidines are quite efficient radiosensitizers, although the mechanism by which very small amounts of, for example, IUdR in the DNA render cells increasingly radiosensitive is not known. Finally, the possible carcinogenic role due to 'miscoding' DNA analogues has to be borne in mind.