

2-⁸³Br-2-deoxyglucose, 6-bromo-glucose: two Trojan horses for the war against cancer? – 3-¹⁸F-pyruvate as a probe for enhanced expression of monocarboxylate transporters in cancer

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Abstract

After its discovery about a century ago (Warburg), the times fold increase of glycolysis in cancer has become a very studied effect for many years, if not the more, in cancer research, and it is now considered the most relevant hallmark of cancer. So that some researchers have, more recently, proposed inhibiting glycolysis in cancer cells as a very promising cancer therapy. Among the glycolysis inhibitor tested, 3-bromopyruvate, a pyruvate analogue, has soon gained interest among the researchers in this field, perhaps because it was considered, soon after the first studies, quite specific for cancer cells. With the aim of bear some improvement to the therapeutic efficacy of this approach, in this study it is tentatively suggested the use as possible anticancer agents of derivatives of 3-bromopyruvate, glucose or deoxyglucose, some marked with the positron emitting ¹⁸F or the beta minus emitting ⁸³Br, and of a not radioactive glucose, with a Br atom on the C6.

Introduction

It is a well-established fact (indeed, the most relevant hallmark of cancer, for the first time described by Warburg [1], that glucose uptake and utilization are strongly increased in cancer, by 10-fold [2] or more ([3] “and ref. 17-18 therein), due to the overexpression of glucose transporters, especially GLUT 1 and 3 and of the enzymes of the glycolytic pathway ([4] and references 10-11 therein). Oncogenes and mutated or deleted tumour suppressors contribute to the determination of the anomalous cancer metabolism. There are so many papers and reviews that deal with this topic (molecular oncology) that it is impossible to mention them all, so only a few of the papers to appear on this topic and a (quite) recent one review shall be mentioned here [5-9].

Anomalous glycolysis also leads to a corresponding increase of steady state glucose concentration in cancer cells. Deoxyglucose is also taken up and accumulated to a larger extent than in normal cells, as revealed by its ¹⁸F-tagged form in positron emission tomography (¹⁸F-PET). Indeed, it is believed that this glucose analogy is phosphorylated by the glycolytic enzyme hexokinase inside the cell, but not further metabolized; this causes the glucose analogy to remain longer inside the cells, so its localization can be identified by radio-labelling it with ¹⁸F and using positron emission tomography (PET), provided, as seems to be the case, that its time of permanence in cancer cells is longer than that required for the decay of the radioisotope. The procedure has now been employed in cancer diagnosis and treatments for many years, mainly for the identification of cancer metastases.

Of course, since enhanced glycolysis is a fact in cancer, numerous papers proposed the idea of inhibiting it to fight cancer [10-12] to cite only a few.

The idea of attaching a radioisotope emitting beta alpha or gamma radiation to a molecule, preferentially one accepted by cancer cells, like

glucose, for diagnostic or therapeutic purposes has gained popularity in the last few years; of course, the required radioisotope needs to have the appropriate half-life and range, when beta minus or alpha radiation are involved, to allow its massive decay inside the cell, with the consequent energy being absorbed, before its expulsion.

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⁸³Isotope of bromo, ⁸³Br appears to fulfil the above requirements: it emits beta minus radiation with a mean energy value of about 0.9 MeV corresponding to a very low range, (near to that of ¹³¹I emitted radiation used in the therapy of tumour thyroid). Thus 2-⁸³Br-2-deoxyglucose should discharge most of the energy of the beta decay inside the tumour, avoiding dangerous energy dissipation outside the tumour mass. Its half-life is 2.5 hours, thus similar to that of ¹⁸F (110 minutes).

2-⁸³Br-2-deoxyglucose, like 2-¹⁸F-2-deoxyglucose, should be taken up in a shorter time than that of its radioactive decay, and retained inside the tumour mass just long enough to discharge most of its energy inside the tumour. The phosphorylation of the 2-¹⁸F-2-deoxyglucose glucose analogue is thought to be crucial for its retention and it is conceivable that the same would occur for the above halogenated glucose. However, other crucial points should be fulfilled, namely: i) the halogenated glucose must be recognized by cell glucose transporters; this is actually the case, as demonstrated by its ability to inhibit glycolysis [13]; ii) however glycolysis inhibition by 2-Br-2-deoxyglucose occurred at higher concentrations, in terms of the effect on lactate production, as against that of 2-¹⁸F-2-deoxyglucose,

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due to a lower affinity for hexokinase [13]; the resulting competition between 2-⁸³Br-2-deoxyglucose and glucose for phosphorylation may become a problem, in that the required injected dose, to facilitate the above radioactive glucose analogue phosphorylation, could be toxic for normal tissues; phosphorylation of the glucose analogy, as mentioned above, is thought to be important to allow its retention inside the cancer mass. Moreover, a lower affinity of this halogenated glucose for glucose transporters is also possible. As below discussed briefly for the trapping of 2-¹⁸F-2-deoxyglucose, the mechanism involved could be different from that currently hypothesised and thus not depending on competition with glucose for hexokinase.

Despite the above considerations, the problem might be approached first by tentatively evaluating the minimum amount of 2-⁸³Br-2-deoxyglucose that should be taken up by cancer tissues to determine consistent cell death. Determining the correct dose to be injected is not a simple feat and should be done only experimentally, as discussed above.

Now a dose of 10 sievert (currently considered sufficient to cause consistent cell death), should require, in the above discussed conditions (time of permanence of the radioactive drug inside cancer cells to cause complete decay inside, etc.) a final 10⁻⁹ molar concentration inside the cancer mass (assumption 1kg =liter , range of beta radiation = 0,4cm , emitted energy adsorbed in its about entirely). On the other hand, a ten minus, or even much lower, dose in the blood stream and tissues , that is 10⁻¹⁰ molar , should not be toxic at least as immediate effect, as currently reported.

Given the above, the crucial question is whether the dose to be administered to attain such an amount could be toxic for normal tissues, as the ¹⁸F dose would be even larger than that used for the ¹⁸F- PET.

Certainly, the case of 2-¹⁸F-2 deoxyglucose which concentrates inside the cancer mass, as revealed by PET, could provide a datum point despite the recorded difference that suggests a further consideration

Now: 400 MBecquerel is the maximum dose injected for a ¹⁸F -PET . With this data it is then possible to calculate the concentration of glucose radioactive in the blood stream (5.1): Even taking a concentration well over 10 times in the tumour, the above calculation gives a concentration of the marked glucose inside more order of magnitude lower than that of blood glucose.

It seems difficult to understand why such a low concentration should allow a successful competition of ¹⁸F-deoxyglucose for hexokinase against 5-10 millimolar glucose in the blood stream. Consequently, there might be other reasons, such as glucose transporters operating more inward than outward, making the phosphorylation step uninfluential, to explain why 2-¹⁸F-2-deoxyglucose is retained inside the cancer mass, as is the case. Of course this point remain to be clarified.

In the case of 2-⁸³Br-2-deoxyglucose, of course, only trials conducted preferably *in vivo*, but also in cultured cells, could answer the question raised above, by testing any damage found that was caused by 2-⁸³Br-2-deoxyglucose given at the maximum concentration to be reached in the blood stream that does not produce toxic effects on normal tissues. The calculation might be very complex. For example, 20 % of the radioactivity of 2-¹⁸F-2-deoxyglucose is lost in a short time outside the tumour. In conclusion: in the case of 2-Br 2-deoxyglucose, it is not immediately clear, for the reasons mentioned above, whether the injected dose, as accumulated in normal tissues, is low enough to avoid danger to normal tissues while causing consistent damage in cancer cells due to its increased uptake. Only trials could provide an answer to this.

Another major problem is that other tissues, such as the brain, make abundant use of glucose for their energy requirements.

(Non-radioactive) 6-bromoglucose to deliver inside 3 bromopyruvate

For about fifteen years now, 3-bromopyruvate has been studied as a possible, extremely promising anticancer agent, following initial findings that demonstrated that the compound could destroy hepatomas induced in rodents without toxic effect on normal cells [14]. Many studies, in different systems, partly confirmed the efficacy of this drug against tumours, and attempts to translate these results obtained on animals to humans have already been made[15,16] and likely are still ongoing. The cell damaging mechanism of the drug was of course extensively investigated. It was initially suggested that the compound inhibits the hexokinase enzyme [17], thus inhibiting the entire glycolytic sequence and this would be very deleterious considering the fundamental role of glycolysis in cancer (Warburg) .

It was seen, however, that other enzymes especially glyceraldehyde phosphate dehydrogenase [18] were inhibited more efficiently, as later confirmed: the enzyme is really “pyruvylated” [19], the same was true for other enzymatic activities succinate dehydrogenase and others [18,20], thus the drug inhibited oxidative phosphorylation too. 3-bromopyruvate is a strong alkylating agent; it attaches SH groups, by releasing Br⁻, thus “pyruvylating” the enzyme and inhibiting it, especially when the SH group is essential for its enzymatic activity. It has been hypothesized that the drug should not damage consistently normal cells, for monocarboxylate transporters [21,22] are more expressed in cancer cells [23-25] due to the urgency with which the cancer cells need to expel lactate (but inward and outward transporters could be differently upregulated).

Now, it should be hypothesized that I) 6-bromoglucose is recognized by glucose transporters and metabolized into 3-bromopyruvate and thus 3-bromopyruvate should exert its toxic effect, provided a congruous concentration is attained.

Since glucose is taken up in cancer cells to a much larger extent than in normal tissues, and presumably concentrates inside cancer cells, then the release of 3-bromopyruvate in cells could not damage normal cells, but would certainly damage or kill cancer cells, if the dose to be injected is suitably estimated.

Consequently, the first step would be to check whether 6-Br-glucose inhibits certain enzymes in normal cells as 3-bromopyruvate does, and, if so, to determine the IC 50 for these inhibition actions.

H⁺ lysosomal ATPase inhibition is a simple test to run [20] for example in rat tymocytes. After this, the IC 50 for this inhibition effect should be determined. Using an injected dose not toxic for normal cells (thus consistently lower than the IC 50 measured as described above), the release of 3-bromopyruvate in cancer cells would not determine a damage in normal cells, but would damage or kill cancer cells, since glucose uptake in cancer cells exceeds the uptake in normal tissues by many times. To test if a specific concentration of 6-Br-glucose could be toxic in cancer cells, the inhibition of the above enzyme(lysosomal H⁺- ATP-ase in cancer cells ,for example in Ehrlich ascites tumour cells [20] could be measured.

It may be observed that H⁺ lysosomal ATP-ase, glyceraldehyde 3-phosphate dehydrogenase and succinate dehydrogenase all have similar concentration requirement for their inhibition [18-20]

Of course only experimental trials like the one above could decide if this approach could be used in cancer therapies.

3-¹⁸F- pyruvate: a “probe” for the overexpression of monocarboxylate transporters in cancer?

It is generally thought, but never directly demonstrated, to my knowledge, that the specificity of 3-bromopyruvate in killing cancer cells, as largely proven in cultured cancer cells and animal tumours is due to the overexpression of monocarboxylate transporters in cancer, as above pointed out, possibly enhanced, according to some authors, by a low pH [22] such as that found in the environment of cancer tissues [26].

Despite the above [23-25], and perhaps others studies supporting upregulation of specific monocarboxylate transporters in cancer, a more direct approach could be achieved by attaching ¹⁸F, instead of Br, to pyruvate.

To give support to this idea, pyruvate should be ¹⁸F-labeled on the carbon 3. Thus, if the above hypothesis of over expression of monocarboxylate transporters is true, 3-¹⁸F pyruvate could be used, as is currently the case with ¹⁸F-deoxyglucose, to visualize cancer tissues by means of positron emission tomography (¹⁸F- PET). This could be used to determine, eventually, if the conditions to enhance the metabolite uptake exist.

Of course, it is crucial that, like 3-Br-pyruvate, 3-¹⁸F- pyruvate be transported inside the cells, and this is likely, thanks to the smaller size of the F atom (lower steric hindrance).

3-⁸³ Br pyruvate: a carrier for introducing beta radiation in cancer

As the work mentioned above indicates a well differentiated uptake between cancer and normal tissues (ratio of uptake/kg in cancer to the same in the whole body higher than 10) then substituting ¹⁸F with the radioactive ⁸³Br in the pyruvate molecule, in an attempt to introduce a radioactive isotope emitting a beta minus radiation, preferentially in cancer cells, with the expected toxic effects, could be taken into consideration. Of course, as described for the test with 2-¹⁸Br 2-deoxyglucose, the uptake in normal tissues should not be toxic for them.

This could help overcome the issue that there are other tissues that make abundant use of glucose, such as the brain and others.

Concluding remarks

The approaches suggested in this study should be practicable, provided that 2-⁸³Br-2-deoxyglucose is taken up as does 2-¹⁸F-2-deoxyglucose and accumulates in cancer tissues at least ten times more than in normal tissues. Inside cancer cells 6- Br- glucose is up taken and processed like glucose, namely, it is metabolized to 3-bromopyruvate

3-¹⁸F-pyruvate is up taken like 3-bromopyruvate by cancer cells and does not lose more of its radioactivity by releasing ¹⁸F outside the cancer cells.

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