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A rational approach to the systemic treatment of cancer involving medium-term depletion of arginine

Review Article

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Summary

Arginine catabolizing enzymes have been applied to cancerous material for over 60 years. The scattered reports in the literature during this period have been reviewed on several previous occasions. This article will be concerned with reports mainly over the last 6 to 7 years on the ability of arginine catabolizing enzymes not only to inhibit proliferation, but to kill tumour cells. The selectivity of action is based on the inability of many tumour cells to circumvent arginine deprivation by utilizing (recycling) various precursors available through the urea cycle. While this offers an immediate window of opportunity for treating melanomas and hepatocellular carcinomas in particular, *in vitro* treatment can be customized so that even those tumour cell lines with intact urea cycles can be targeted, making the protocol more generally applicable. Since i*n vitro* studies have provided convincing evidence of the efficacy of arginine degrading enzymes, and animal tumour models responded similarly, this treatment has moved on into clinical and veterinary trials. Initial findings are encouraging, which could be effective with many tumour types, from leukemias to melanomas. This is made even more attractive because arginine deprivation protocols can "stage" tumour cells for combination therapy where cells have not been killed outright by deprivation. This is also selective because deprived normal cells will have become quiescent but soon recover on restitution of the missing nutrient, whereas tumour cells in cycle can be hit by low doses of cycle-dependent cytotoxic drugs.

I. Introduction

Rational new approaches to the treatment of cancer

Although gene therapy might eventually become an excellent means of approaching cancer treatment in the future, it will need to run alongside other conventional procedures (radiotherapy, chemotherapy and "biotherapy", to name three other approaches). It is likely that no one modality will do the job, and in this article my intention is to draw attention to a form of therapy that can work on its own, but which will almost certainly help in getting cancers into a state that will greatly assist in their demise by these other modalities. It will deal to some extent with genes, but only those related to citrulline to arginine metabolism in the urea cycle. But, because we will be discussing an improved approach using enzymes that was initiated over 40 years ago, the rational for its development has to be assessed in comparison with conventional therapeutic procedures in this section.

A. The promise of an anticancer drug

The biggest fear with cancer is that, if cancer is not diagnosed early, prognosis gets worse with time. Late presentation often leads to the tumour being considered

untreatable, which is undoubtedly a problem that faces many clinicians unless greatly improved screening procedures become available. As far as "novel" approaches to treatment are concerned, cancer vaccines are back in vogue after being championed in the early 1960s, but now improved "tumour-specific" vaccines are being developed (Kaplan, 2004). With regard to other types of immunotherapy, a general mechanism may otherwise need to be attacked, such as angiogenesis, especially where it is "induced" by a developing tumour (Hou et al, 2004). Genetic engineering still presents too many attendant problems to deliver significant benefit in the immediate future, since procedures themselves could be carcinogenic. While there is little evident success with several other *innovative* approaches to cancer treatment, few would disagree that, in general, protocols for treating cancer are improving through trial and error as much as through rational approaches, photoactivation of drugs *in situ* being a good example.

However, experimentalists no less than clinical oncologists really do want to see treatments that are better targeted not just generally aggressive to both healthy and unhealthy cells. Designer drugs that block receptors of key growth factors are being developed, but cancer cells are adept at circumventing them; and we must consider how normal cells are affected by them. Chemicals continue to be discovered that may have anti-cancer effects, but very few pass muster in the way that tamoxifen and cisplatin have.

For want of something better, many patients continue to receive some cocktail of the old and more familiar drugs largely arrived at through experience rather than any particular strategy. Like traditional Chinese medicine, patients get appropriate mixtures of extracts (=drugs), but in refined proportions and given in a seemingly logical time-sequence. Combination of drugs and/or other modalities is fashionable, using radiotherapy, hyperthermia, "biotherapy", gene therapy, and immuno-therapy along with conventional anticancer chemicals. They may do better in future, but that calls for a truly rational basis for the various complementations rather than some empirically discovered concoction, since time is not on the oncologist's side. So, is the problem that we continue to fall back on old drugs because we do not persevere long enough with new ones to find ways of optimising their efficacy and specificity, both alone and with other modalities? If this is the case, what difficulties have to be surmounted to achieve more effective use?

Before any further considerations, a word is needed about our vocabulary when referring to cancer treatment - one that reflects an ingrained philosophy. I have already referred to an "armoury" of drugs. We "attack" cancer as we would an alien; we use "weapons" and seek magic "bullets", much as Paul Ehrlich was after in the early days of anti-infective agents. The philosophy probably reflects certain expectations, namely that we:

a) usually seek not just good, but spectacular results

b) are invariably impatient for quick results, and

c) want to continue believing in a panacea, or some basic protocol, that can be applied to *all* cancers.

B. A vain hope?

With bacterial infections, the "panacea" used to be antibiotics, and with cancer it would be wonderful to have a generally effective agent (or class of agents) with which to treat "cancer". But we know this does not apply to tumours because they are neither foreign invaders, nor are they likely to respond in any one way to treatment. Miss G's ovarian carcinoma may look like that of Mrs H, but in fact it is a different unique tumour, and hence there is no obvious reason why it should respond to the same treatment. Furthermore young Miss G has a distinctly different metabolism, immunological composition and hormonal milieu from the older Mrs H. Indeed, the hosts can be physiologically less "similar" than their tumours. Another compounding difference is that Mrs H has a strong positive attitude to her condition, whereas Miss G has rather given in to hers at an early stage. This example alone should quell any notion that there is a "common" cure for cancer; and we should certainly not be using the singular, just as it is quite inappropriate to harp too often on the word "cure". It is *not* that we have been unable to cure some cancers (choriocarcinoma being a early example of excellent response to chemotherapy with monitoring of hCG). The perception seems to be that, *since we can cure some cancers, then in theory we should be able to cure them all.* If this mindset persists with doctors and researchers, it will rub off on cancer patients, giving many false hopes.

While being more guarded with the use of the word *cure*, let us nevertheless heartily rejoice when occasionally complete regression and freedom from the disease is achieved. For the overwhelming majority of cancer sufferers, the best we can hope is that their disease can be brought under "*control*", and that good quality of life can be maintained for as long as possible. In his recent article, Kitano (2003) referred to the robustness of cancer in evading therapeutic interventions, since new clones of mutant tumour cells arise with increasing drug resistance. By gaining insight into the feedback controls that have been usurped when resistance develops, he believes that a more subtle "systems-level strategy" in cancer treatment may be feasible. As noteworthy as his concept is Kitano's persistent use of the word "control" rather than "cure".

C. Beyond the pale?

Because we are going to discuss a strategy that is systemic, we need also to mention cancer cases that are diagnosed too late and are seen as untreatable. These are the very ones that we have been exploring most diligently, since we delude ourselves if we think we can treat cancer patients *en bloc*, using treatments that seem to have given some positive benefits overall from clinical trials. This orthodox practice becomes little more than a weak averaging system that takes little consideration of the uniqueness of each cancer *and* host (as discussed above), undermining a holistic approach to the treatment of malignant disease.

D. Rational approaches to cancer therapy

We are often left with blitz-like approaches to advanced cancers, designed to kill the tumour while

hopefully managing to keep the host alive; such treatments can be devastating to the patient. The most obvious targets are malignant cells with high and persistent proliferative impetus (Strauss et al, 1995), constantly replicating their DNA, and these cells are capable of wandering off, thereby populating local and distant tissues. But this can equally well describe the behaviour of many stem cells required to repopulate the normal drop-out of cells from healthy tissues. While we have learned an enormous amount about the cell cycle and its regulation (Murray, 1992), we should not delude ourselves that our present understanding remains other than sketchy. Cdk2 and cdk4 knockouts have not proved lethal, and there must be many interlacing checkpoints and escape routes that allow proliferating cells to get round difficulties at supposed checkpoints (Otetsu and McCormick, 2003). Nevertheless, the cell cycle must remain one of the basic guiding principles of all rational treatments.

The essential question is: has there been any real advance in the *selective* control of cancer? Where are the magic bullets promised in the 1970s? If growth factor receptors can now be blocked with designer analogues, why do they remain effective for only a short time? It may well be that the cell cycle paradigm alone is simply not good enough, and needs to be supported by adjunct strategies. And this is undoubtedly where statistics of recurrence rates indicates most success in recent years. In addition, if we had greater understanding of *in vivo* tumour growth kinetics, matters would undoubtedly improve. This article is concerned with these problems and presents a new approach that may help us to gain far better *control* over a spectrum of cancers than hitherto.

The best lifeline to more specific approaches is undoubtedly through a better understanding of genetic changes associated with malignancy, where the identification of clusters of genes associated with a high cancer disposition and those with frank tumour development lead to a more predictable scenario. Combined with the impact on the genetic expression of these cells in relation to their cell cycle characteristics, there is still hope that more subtle approaches to controlling individual cancers can be devised. Let us acknowledge that cancer treatment continues to improve slowly as we succeed in piecing together the genes and their products that control proliferative potential and activity within every cell population at risk to cancer. Let us also acknowledge that there is indeed some fundamental difference between normal and cancer cells within the body, and some inappropriate behaviour within tumour-altered stroma (Mukaida et al, 1991; Maffini et al, 2004). Hence we have always to focus on the fundamental differences, and I will discuss now how we might exploit them to better advantage using some new anticancer "drugs" that are also natural enzymes.

II. Control of growth and the cell cycle through manipulation of the availability of essential nutrients

A. Choice of an amino acid

The body requires many nutrients, especially for protein synthesis. We turn over some 400 grams of nitrogen per day in protein replenishment. Since cells are constantly dying, neighbours or stem cells must divide and grow to maintain mass. Inevitably some can go wrong and can become potentially cancerous. Surveillance can keep the number of aberrant cells down, but cancers can still arise where aberrant cells evade detection. (It is often remarked how astonishing it is that cancers do not arise much more frequently in organisms like man composed of so many billions of cells. In fact, every man who becomes a centenarian will have or have had a prostatic lesion histopathologically gradable as a tumour.)

For cells to grow, whether malignant or not, nutrients have to be in constant supply, and the twenty or so amino acids making up proteins are of particular importance. Seventy to 80% of body dry mass is protein, and therefore there is a high and continuing requirement for amino acids, of which we need about 11 amino acids in our diet that our bodies cannot synthesise.

Our own studies (Lamb and Wheatley, 2000; Wheatley et al, 2000; Wheatley and Campbell, 2002b) were preceded by work indicating a high requirement for arginine by tumour cells 50-60 years ago (Bach and Lasnitski, 1947; Bach and Simon Reuss, 1953), and led to Bach and Swaine (1963) noting that rat tumours responded quickly and impressively within 4 days to deprivation of arginine. Since those observations, very little *concerted* work on arginine manipulation has been done until the last 6-7 years, probably because the early work was eclipsed by the advent of powerful anticancer drugs in the 1960s, the mustards, the nucleotide analogues, and other "bunkerbusters" of the modern therapeutic arsenal. Also, the somewhat isolated findings of Storr and Burton (1974) had already sown seeds of doubt that amino acid manipulation held any particular promise. But this was based on the idea that tumours had a particularly high requirement for arginine, which was an empirical observation that was only partially true or relevant. Herein lies the Achilles' heel. If growth is dependent on an adequate supply of all the amino acids, then if one becomes limiting and cannot be made any faster by the body [a non- or semi-essential amino acid – for a better understanding of this anomalous nomenclature (Inglis et al 1984) – becoming an essential one], it needs to be supplied in the diet. This *extra* "demand" was what the early researchers noted with arginine. We do indeed hold tumour growth to ransom by controlling arginine. But the outcome is even better than that, because tumour cells have in many cases:

1. lost the ability to make arginine from citrulline (Philip et al, 2003; Wheatley and Campbell, 2003),
2. stay in cycle instead of moving out of it into G1 or G0 (Scott et al, 2000),
3. die within 3-4 days in many cases, probably as a result of trying to cycle when insufficiently resourced, and without any further intervention (Wheatley et al, 2005; Scott et al, 2000)
4. because they stay in cycle, they continue to be suitable targets for cell cycle-dependent cytotoxic agents (Wheatley, 2004), whereas normal cells become quiescent and relatively resistant.

If we can maximise our exploitation of these factors, there is a very good chance that a relatively significant proportion of tumours will respond in the way we would wish, allowing them to be brought under stricter control. And this would apply not just to local tumours, since the strategy will be equally effective in tackling terminal and widely disseminated disease (Leung and Johnson, 2001).

Of the twenty or so amino acids that could sensibly be manipulated (by deprivation), arginine was the first choice, although others had been quite extensively investigated, including methionine, tryptophan, and phenylalanine (Wheatley and Campbell, 2002a). One reason is that arginine is required in high concentration to sustain tumour growth in animals (Bach and Lasnitzki, 1947). Another is that arginine features in a plethora of metabolic pathways (**Figure 1**), and extensive work on most amino acids has led us to accept that although this presents a number of complications in body metabolism, arginine remains the best choice because of its position in key pathways, especially those relating to the urea cycle. Kondoh et al, (personal communication) emphasise this point because they found that cells stressed by nutrient deprivation regulate arginine transport through ATR6 downregulation of the y+ carrier of arginine in the cell membrane. However - and paradoxically - G0 arrest is dependent on having a tiny amount of arginine available because complete deprivation would not permit cells to reach this point of arrest. Scott et al, (2000) found that arginine deprivation did not generally arrest most malignant cell types in G1/G0 cells, but that they remained in cycle and tried to continue, inevitably leading to imbalanced growth, and for as yet unexplained reasons resulting in the early demise of cells of the more malignant phenotypes *without any further intervention*. This was an unexpected bonus, but does not occur in all tumour cell types, since those that can exit the cycle can survive the period of deprivation more like normal cells. But in addition to this we have since found that in many instances tumour cells have lost the ability to convert citrulline into arginine, making them particularly vulnerable because they have no recycling capacity (Wheatley and Campbell, 2003; Wheatley et al, 2005).

It was primarily these ideas that led us to believe that if we could indeed control arginine availability, we might get much better control over malignant growth.

B. An approach based on deprivation coupled with the differential cell cycle dynamics of normal and tumour cells

For any treatment to bring cancer under control, there are two major desiderata. The first is to stop unlimited growth of the tumour, and the second is to reduce to a minimum the dispersion, seeding and early development of metastatic deposits. Unless tumour cells have some flagrantly obvious determinants on their surfaces that are not shared by any other vital cells of the body, the problem is going to be difficult, but not necessarily intractable. To achieve both aims requires that the treatment given is systemic so that it reaches all parts of the body, even the deepest capillary beds and lymphatics vessels. It also needs to be tolerable during the time it is being effective.

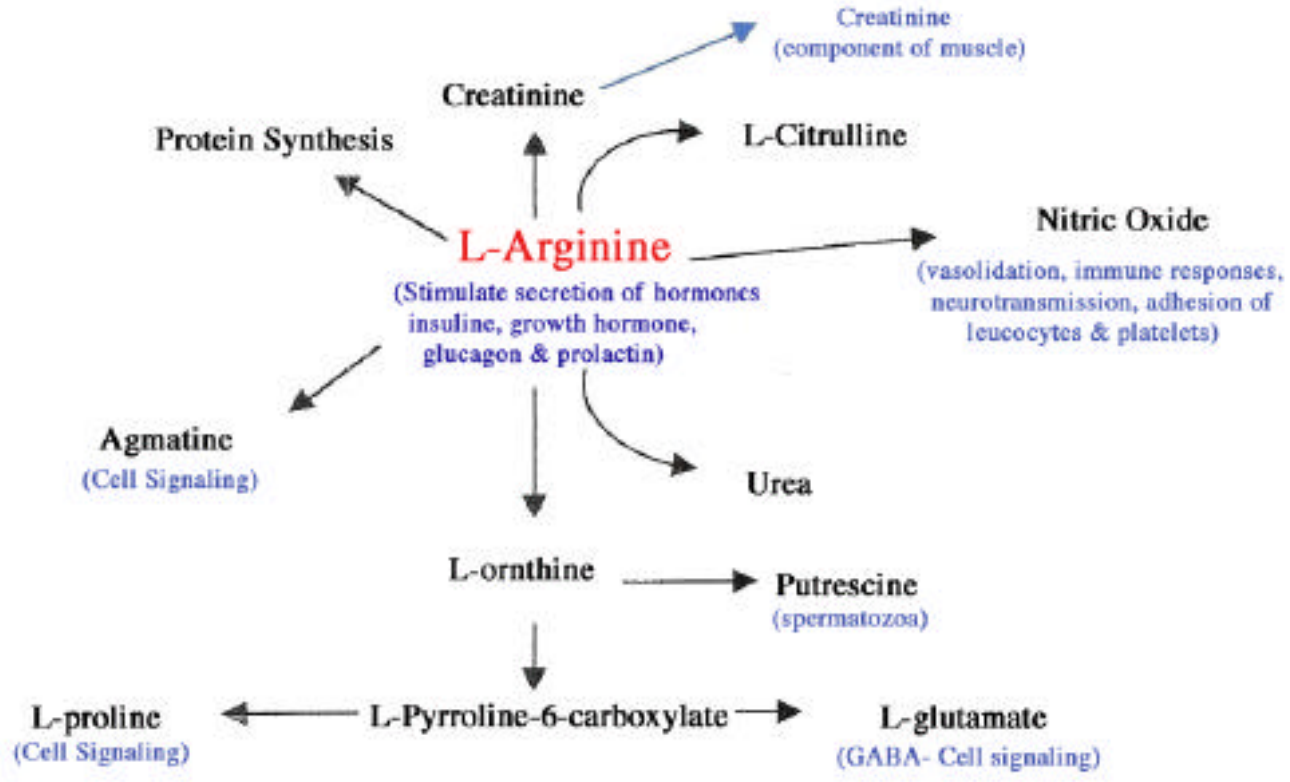

Figure 1. Scheme of the main pathways of involvement of arginine in cell metabolism

Whole body irradiation followed by bone marrow transplants can save mice from certain death, but the same procedure carried out on a human patient is going to be very much more complicated. And the risk of subsequent carcinogenesis for a long-lived species would be greatly increased. But much the same tactic has been used with flooding (overdose protocols) in chemotherapy, which essentially will kill the tumour and the patient, with the oncologist hoping to have the skills required to "rescue" the patient when the tumour has receded.

The main difference between tumour and host tissues is generally considered to be the incessant proliferative activity of the former. But crypt cells of the gut are not cancerous and they are persistently proliferating. A clearer statement of the underlying difference between tumour and normal cells *is that the former keep growing under circumstances where the normal cells would arrest* (Tepic and Pyk, 1994). They have lost an element of control, an inhibitory control that should make them quiescent when circumstances dictate it (Soto and Sonnenschein, 2001) than have a green signal on all the time that goads them into incessant growth (Raff, 1992).

III. Procedures

A. Introduction

Let us now look briefly at the various methods of deprivation. Much work has been done *in vitro*, since removal during formulation (combined with dialysed serum) is easy. We found that all three catabolic enzymes of arginine are effective at ~1-2 units per ml medium. But *in vivo* it is a problem of a different order of magnitude. Leaving out arginine from the diet achieves very little if anything in most animals except when the amino acid is required in large amounts, e.g. during embryonic and early neonatal growth. Reducing arginine in the blood by dialysis has been tried, but poses many (although not insurmountable) technical problems. But we have also shown that all three catabolic enzymes of arginine are effective here as well. The various procedures and variations upon them *in vitro* and *in vivo*, will inevitably be different, but the goal is the same - to lower arginine availability to about the micromolar level (Scott et al, 2000) for long enough to eliminate the majority of tumour cells – usually about 4 days, and therefore an effective "window" would best be about 7-8 days, which is why we have used the expression "medium-term" in the title**.**

The main drawback with any such procedure is that the body's homeostatic mechanisms are very potent in maintaining consistent plasma levels of amino acids. Therefore one has to devise methods of reducing this counteraction.

B. Diet

Where an (essential) amino acid can be reasonally controlled by diet, e.g. phenylalanine, dietary means may work to some extent (Lorincz and Kuttner, 1965; Wheatley, 1998). In contrast, an arginine-free diet achieves very little on its own it, but nevertheles diet has be considered because it is a prime source of replenishment. A,low protein diet will clearly reduce uptake and assist in keeping plasma levels lower than is a normal diet is given.

We also need to control other sources of arginine that would easily circumvent an attempted deprivation, since other facets of homeostatic control are also very powerful in the body. One source of arginine comes indirectly from the gut, where cells can synthesise a lot of citrulline, so dietary restriction makes sense. In addition, the bacteria of the gut will synthesise many amino acids, and so a purging of gut bacteria with antibiotics provides another step to the preparation for treatment. Protein turnover also has to be reduced, and this is aided by resting the body state, in combination with treatments that slow protein breakdown (now with certain drugs, but also with hormones – see below).

C. Dialysis

While dialysis offers nothing new regarding in vitro work because arginine can be controlled by formulation and the use of dialysed serum, it has provided useful information on the overall *modus operandi*. Cells generally have to be free of any citrulline because many lines can convert this to arginine. In vivo, dialysis is complex and in the Shettigar patent (1990), the extracorporeal loop includes low molecular weight filtration into chambers where the inner filter walls have arginase attached. The arginine passing through gets broken down to urea and ornithine, and these breakdown products pass back into circulation. Arginine can be lowered, but not usually to "therapeutically desirable" levels. Ornithine will be recycled and provide more arginine, and since citrulline is not controlled, homeostasis is scarcely touched. In trying to improve this system, Tepic and co-workers (see Scott, 1999; Cambpell, 2004) have proved that *open circuit dialysis* can be much more effective. This purges the blood of all low molecular weight compounds (~10,000 Da). The returned blood requires many additives, but arginine, ornithine and citrulline are omitted. Glucose and insulin are reinfused using a clamp, and the insulin is raised in concentration to act as an inhibitor of protein catabolism. It is possible to go <5 micromolar blood arginine for several days of continuous dialysis, and 1 micromolar levels have been achieved. Dialysis brings about other changes that have been considered complications, one being vascular tone in the absence of arginine, and another the maintenance of effective levels of thrombocytes because many come out of circulation on the dialysis filters. Prostacyclin and other treatments, e.g. sodium nitroprusside as an NO generator, have been used to reduce these problems. The effects of hypovolemia must also be avoided. Dogs receiving dialysis can be kept many days on almost continuous dialysis, and some tumours show good resolution during this period. To ensure low protein breakdown (and in addition to the insulin treatment), animals are given antibiotics to clear the gut of amino acid synthesis by bacteria, the diet should contain very little protein, and muscular activity has to be reduced so that the peripheral circulation is slowed. There is no reason to suppose in principle that this technique would not work as well in human beings, although such a move has yet to be taken.

However, because of the intensive nature of such a procedure, simpler alternatives were sought and a return to enzyme treatment on its own has been the main direction in the last few years.

D. Enzymes

Finding doses of catabolic enzymes that were effective in vivo is a different matter, and has to be done empirically, but simply providing enzyme in the blood stream is too simplistic an approach because of its half-life and immunogenicity. Bach and Swaine (1965) had started by using bovine arginase on tumours in rats. The enzyme also has to be kept at the highest possible specific activity for as long as possible, and as a (foreign) protein it needs to be protected from rapid proteolysis.

Work in culture has shown that, whatever the source of enzyme, as long as arginine is reduced to the micromolar level, many cancer cells will die, while normal cells recover from quiescence when enzyme is removed. The sources of enzymes can be the following:

1) purified bovine, dog, or other - i.e. animal, with carnivore livers being vastly richer in arginase than herbivores)

2) human arginase released from the liver *in situ* by trauma (see below)

3) recombinant arginases produced in bacteria by transfection (Buch and Boyle, 1985)

4) enzymes prepared from plant or microbiological organisms, notably arginine decarboxylase from plants (Ikemoto et al, 1990) and the more popular arginine deiminase from *Mycoplasma arginini* (Miyazaki et al, 1990; Takaku et al, 1992; van Rijn et al, 2003, 2004; Ensor et al, 2002; see Wheatley, 2004 for a review).

The important features are (a) to have high specific activity; (b) low immunogenicity; (c) long half-life in the blood or peritoneal cavity (with adequate co-factor levels). (a) This depends on production methods, but can be very high (Buch and Boyle, 1985) in some recombinant preparations. (b) Immunogenicity does not often seem to have been a problem, although reactions to repeated treatments can be seen in highly sensitive animals, such as guinea pigs, that can tolerate several treatments before showing distress (Bomalaski et al, 2004). This and more direct toxicity are not severe problems, but crucially "pegylation" (covalent attachment of polyethylene gycol (PEG) of M_R 5,000 or 20,000 to lysine residues of the enzyme, the procedure introduced by Savoca et al, (1984) helps to mask the enzyme. The 20,000 PEG tails probably give the best compromise (Bomalaski et al, 2004). (c) The bonus here is that the same pegylation gives a much longer half-life *in vivo* with less compromising (loss of specific activity) of enzyme functioning. Periods of exposure show some enzyme persisting for days, which by regularly topping up can keep arginine levels very low. With enzyme production going into production mode, sufficient stocks of potent enzyme preparations are now available to treat human cases, and this has already been reported (Curley et al, 2003; Izzo et al, 2004).

There are other ways of releasing arginase to depress arginine in the blood, and one procedure used by Cheng et al, (2005) involves transhepatic arterial embolism. Liver damage is diffuse, and arginase leaks out in abundance with arginine levels plummeting for at least 2 h. Even this seems to be enough to bring about a sudden change in tumour progression, and as yet the reasons for the effect persisting long enough in some patients to cause quite long regression is not understood. Irrespective of the reason, the result is what is important. This procedure can have a useful place. It should also be remarked that, in accord with recent *in vitro* data, once arginine deprivation has done its main job, tumour cells unlike normal cells remain vulnerable to other treatment, *specifically cycle-dependent drugs*, such as hydroxyurea (Wheatley, 2004). Combination therapy is an obvious way of "cleaning out" more tumour than arginine deprivation alone can achieve. We refer to this process as "staging" the tumour cells ready for the next modality, because they ought to be vulnerable whereas normal cells are quiescent and less likely to be affected.

E. Requirement for more experimental work in cell culture and *in vivo*

It is important that such clinical advances are accompanied by experimental work. Our recent studies (Wheatley and Campbell, 2003; Wheatley et al, 2005) – which can go on as an open-ended survey, especially with tumour biopsies rather than designated normal and tumour cell types – shows tumour lines that are most vulnerable and those which can utilise citrulline easily and be relatively resistant. Since arginine catabolising enzymes in the circulation will destroy citrulline released from the gut-kidney axis when it is converted to arginine, there is no problem that many types of tumours ought to be vulnerable, even if they could utilise citrulline. But destruction of substrates is not instantaneous and therefore tumour cells that have no ability to utilise citrulline are going to be most vulnerable. The ability to carry out the conversion depends on the urea cycle enzyme, argininosuccinate synthetase. There is a good correlation between the presence of this enzyme (or its mRNA) and the ability of cells to use citrulline (Miyazaki et al, 1990; Takaku et al, 1992; van Rijn et al, 2003, 2004; Ensor et al, 2002; Sugimura et al, 1992, 1992; Campbell, 2004; Dillon et al, 2004), and the discussion in Wheatley (2004) can help to guide us into assaying the status of tumours (and hosts) before selecting the appropriate treatment. This, however, could also be the route – through an inducible argininosuccinate synthase pathway – to resistance, unless citrulline-arginine conversion is always held in check (Shen et al, 2003).

There is no question that we also need to see more experimental tumour work in animals to resolve some of the quite disparate results seen in the early work from Bach's group with that of Storr and Burton, (1974)**.** Why do the new enzyme treatments seem to work better than those seen before, although the initial results of Bach and Swaine, (1965) were quite dramatic in such a short treatment time using bovine arginase? There is no question that a lot more needs to be learned about the arginine requirement of not just growing tumour cells, but also normal cells. For example, all our studies on established kidney cells, showed little ability to convert citrulline into

arginine in culture; yet in the body they must do so. Too few studies on rodent have been done, but in one shortly to be reported (Cheng P, personal communication), the ability of arginase to cause regression of Hep3b cells in DBA/2 mice will be shown to be quite remarkable. After several weeks from the time enzyme stopped the tumours regrew, but clearly a single episode of enzyme treatment with human recombinant arginase must have reduced the size to close to a critical tumour inoculum for this length of time to elapse before the tumours began to reappear.

IV. Concluding remarks

The above overview has been too limited to reach the depth of information required for many scientists and clinicians to see that arginine deprivation will have a major impact on tumour treatment. Much more consolidation of the work needs to be done in many more centres. There are elements of the overall procedure – control over body arginine levels – that will be understood by many, but there remain other findings that are difficult to interpret. As an example, I mentioned that arginine deprivation would compromise vascular tone. Indeed, in large animals it may. But in small laboratory animals it does not seem to do so. When it is appreciated that a very small amount of NO is sufficient to keep tone in vascular endothelial cells, and that in these cells arginine is not the first requirement for the eNOS but citrulline, one sees that the body has used a compound that it will always be making and is not in short supply to generate *in situ* by direct channelling arginine from citrulline using argininosuccinate synthetase and argininosuccinate lyase in the endothelial cells (Pendelton et al, 2002). eNOS only uses nascent arginine generated in this way, and is not affected by big changes in arginine availability from the blood (Shen et al, 2005).

The future situation looks even more exciting when one considers that the advent of metabolomics will be most useful in arginine studies of this nature, especially after deprivation and with so many differences in the ability of human being and their tumours to metabolise arginine, citrulline, argininosuccinate, ornithine and other associated amino acids (not to mention the involvement of aspartate, urea, nitric oxide and many other metabolites). This could be very helpful in diagnosis as well as in the control of treatment. Much more than simply the levels of blood arginine and ornithine can be followed.

As mentioned in my introduction, it seems we do in fact have a new modality for cancer treatment. It is by no means fully rational because we do not understand many of the elements mechanistically, least of all how tumour cells die without any further intervention when arginine is suppressed; but at least it works. Surely we can build on an idea which now has been around for over 60 years that by controlling arginine availability, we may get some hold, if not some *strangle*hold, on tumours. The technical expertise has been developed, but we have a long way to go before an *optimised* basis for cancer treatment will be available. In addition, it offers a means of "staging" tumours for treatment by other modalities. Here again, some appropriate cases need to be identified where we can bring tumour cell numbers down throughout the body (in widely disseminated disease as well as local tumours) to below a critical mass that can remain under further control, just as is successfully achieved with lymphomas and leukemias.

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