jnm/ EDITORIAL

Of Models and Men

THE HISTORY

Somewhere in the dim remote history of our species there probably lived a caveman whom I choose to call Og. He was an inventive, curious fellow and prepared many potions for his fellow cave dwellers in the community of Gronk. One day he cut his toe and the pain was so bad he sipped one of his own concoctions. The next morning the toe was symptomatically better, and over the next few days it improved greatly. Without further studies he published the results on his walls (*1*) and invited his colleagues to visit. There was a great deal of excitement and the brew (which he named Nog) was, without further testing applied to cut toes.

At first it was a panacea and business boomed; then people began to notice mediocre results. With the passage of time, more problems were observed and finally, Pog, Sog, Mog, and Jog studied Nog in their sabre-toothed tiger and dire wolf models and observed no difference between the control and experimental animals. Nog fell into disrepute and was finally discarded. Two years later Og noticed a peculiar mold growing in a corner of his cave which seemed to help heal infected cuts. He published that, too, but no Gronkonian ever attempted to reproduce his results since Og's credibility had been destroyed.

Several millennia have passed since our scientific ancestor "blew it" in the land of Gronk. Technology has changed, but not the human spirit, which appears at time to be composed of at least 50% impatience. Thus, in 1974, Tc-99m stannous citrate, with a minimum of published animal testing, was declared by a pharmaceutical company to attain "high and selective uptake" in neoplastic tissue (*2*), and, furthermore, to be free of false positives and false negatives when used for brain and bone scanning. These sweeping Ogonian statements got the attention of several of our colleagues (*2-6*).

Benes, Heinzel, Marwik, and Opperman (*3*) found it to be extraordinary for brain tumors, bone tumors, and metastatases. Uptake with the lesion was said to be high.

Kempf, Migueres, Jover, Lenet, Guiraud, and Lespinet (*4,5*) reported on 75 patients, 45 of whom had pulmonary malignancies (the remaining 30 had benign lung disease, including inflammation). They detected all 45 malignancies and four benign diseases. They stated, however, that Tc-99m stannous citrate could not detect small lung lesions ($\times 2$ cm), was poor in the mediastinum, and was of limited use in detecting metastases.

Lundell and Casseborn (*2*) found it of no utility in differentiating benign from malignant thyroid neoplasms, and it clearly missed a bone metastasis that occurred in one patient with follicular carcinoma. Lundell, Garmer, Casseborn, and Ruden (*6*) then compared it with Tc-99m diphosphonate and conventional roentgenographic techniques in the detection of bone metastases, and it came out last. In other work by the same group (*6*), Tc-99m stannous citrate was found to accumulate in a fracture model, which hardly indicates tumor specificity.

Hunt, Maddalena, and Bautovich (*7*) compared Tc-99m gluconate, Tc-99m stannous citrate (solcocitran), and Tc-99m tartrate in an osteosarcoma model and a solid Erlich carcinoma model. The best tumor/bone ratios obtained in the osteosarcoma model occurred with Tc-99m gluconate because that compound had the lowest bone uptake. All three drugs were said to concentrate in the Erlich's tumor better than in normal tissue. Unfortunately, the absolute values of the above are unknown to this author since only an abstract is available.

In this issue of the *Journal*, Tc-99m stannous citrate (Solco Basle Ltd., Switzerland) has been studied in another animal model (*8*). This is not one recently pulled off the shelf; rather, Drs. Haynie, Konikowski, and Glenn have developed in their methodical manner, a useful tool for evaluating the imaging potential of a radiopharmaceutical in CNS tumors (*9-11*). They have accomplished this by again and again using their model to compare compounds and observing that similar results occurred in the human. While they have previously indicated that one cannot transpose *numbers* from animal to man, they correctly suggest that one can transpose *concepts*. There is more than a little evidence to support this hypothesis. For example, Ga-67 has been studied in several tumor models (*12-16*). If one compares the quantity of

uptake by these malignancies and the tumor/background ratios reported by the various investigators, wide differences are observed. On the other hand, when the results with Ga-67 are compared with other compounds *within the same model*, Ga-67 usually proves to be one of the better ones. It is this hierarchy that seems to be useful. The absolute values frequently differ, yet the relative values are similar.

Our group also has studied solcocitran using a thigh-implanted, strain-7777 Morris hepatoma model (*16*). The results were strikingly similar to those observed by Haynie, Konikowski, and Glenn (*8*). The blood clearances were nearly identical, as were the viable-tumor/blood ratios. Furthermore, the percentage of the dose accumulated within the tumor was a small fraction less of that found in the same model for Ga-67. The animal data therefore correlated best with the observations of those investigators who doubt the human tumor specificity and clinical utility of solcoitrans.

THE ENTITY

What kind of animal data, then, would indicate a compound to be a superior tumor-scanning agent, and where do technical problems lie that might return inaccurate data from such a model? Let's deal with the pitfalls first.

The tumor should not vary greatly in size within a data-point group—or for that matter between studies. As malignancies enlarge, their blood supply frequently becomes erratic, with unequal tracer distribution in the primary. Large tumors may also act as a sump, decreasing the quantity of tracer usually extracted by other organ systems and thereby giving a false impression of the overall pharmacodynamics.

Another problem of fast-growing tumors is their tendency to become necrotic, and as has been shown (*16,17*) the presence of necrotic tissue in the specimen can produce bizarre results if one is not careful to distinguish dead from viable tumor.

The animals themselves should be about the same size. While the percent of the tracer dose extracted by the entire liver of a 100-g animal and a 200-g animal might be the same, the percent of the dose extracted *per gram* by the smaller liver will be much higher than by the larger. This plays havoc with the tumor/background ratios, which are usually figured on the basis of percent dose/g. Efforts to correct for this mathematically (*18*) have been developed but, in fact, are not widely used.

Random variation is another problem, and it can be overcome only by volume. In most biological data eight to ten animals per data point are preferable for analysis by the Wilcoxan or Student's t-test. Fewer than four animals per data point render evaluation by these tests useless regardless of what they indicate. In general, five or six animals per data point are a minimum for meaningful analysis.

Finally, one should carefully observe the tumor model for change when using it through many generations. Tumors noted for metastasizing early—and to a specific organ—may quit doing so. A tumor's growth rate may change or it may gradually become more necrotic. Such changes tend to make recent data difficult to compare with those accumulated two years earlier. A good way to ensure the reproducibility of your model is to store a goodly quantity of tissue from the first few tumor transplants in a cryogenic chamber; then, if problems arise, it is simple to reestablish the original cell line.

So much for the problems; now, what parameters make a model a seer? One thing of considerable importance is the absolute quantity within the tumor. If one were imaging rats only, this could almost be discounted, since the whole organism rarely weighs more than 500 g, and a 10-g tumor would represent 2% of the total weight. If it extracted 0.1% of the dose per gram, the total amount in the tumor (1%) should be easily detected. In a 70,000-g human, however, the 10-g tumor would represent 0.014% of the body weight, would get very little of the tracer, and would be buried under several kilograms of nonmalignant tissue. This would make it undetectable or would require scanning for an intolerable period of time. This difficulty was recently brought home to our group after we began clinical trials with I-131 tetracycline. In our model (*16*) this radiopharmaceutical was not acquired in great quantity by the viable tumor and even this amount decreased rapidly with time, yet the ratios of viable tumor to background were usable because of the rapid blood disappearance. Nonviable tumor, however, retained I-131 tetracycline and formed extraordinary nonviable (highly necrotic) tumor/background ratios. Theorizing that some of the cells in a human tumor are dead and should therefore produce high T/NT scanning ratios, we evaluated I-131 tetracycline in patients who had recently been scanned with Ga-67. When the tumor contained necrotic tissue, I-131 tetracycline was as good or better than gallium. Unfortunately, there was not enough necrosis in highly viable tumors to make them detectable.

Close behind the absolute uptake is the question of dynamics: rates of uptake and egress from the tumor and the relationship of both to the falling background. Figures 1, 2, and 3 in Haynie, Konikowski, and Glenn's paper and Figs. 1 and 2 of our own work with I-131 tetracycline and Ga-67 (*16*) demonstrate these phenomena very well. In the case of Tc-99m stannous citrate, the tumor uptake was highest at the time of injection and its rate of egress was not greatly different from that of the blood, brain, or skin. As a consequence, there is relatively little change in the

ratios with time. The same was true for I-131 tetracycline (viable tumor fraction). Gallium-67 revealed a very different pattern *(16)*. Here the peak tumor concentrations were not attained until 24 hr after injection, whereas the blood levels decreased steadily. This resulted in peak scanning ratios occurring at 72-96 hr.

Thus the models predicted not only the *relative* quality of the human scans to be expected (at least where Ga-67 and I-131 tetracycline were concerned) but the time postinjection when the most favorable ratios might occur.

Other predictions are also possible, such as the natural depots of the tracer within the body, difficulties with gastrointestinal activity, the route of elimination, breakdown of the radiopharmaceutical by the tissues, effect of carrier material, and the effect of other drugs on the tracer dynamics. Evidence that the models predict well has been further demonstrated in the case of In-111 bleomycin (*19*), where the ratios at 48 hr postinjection suggested that the label was being leeched off the molecule. In another study Co-57 bleomycin was demonstrated in a model to be superior to the In-111 bleomycin, and human scanning appears to bear this out (*20*).

A PHILOSOPHY

Animal models are not only useful in the evolution of tumor-seeking agents but in the opinion of the undersigned, mandatory prior to an assault with the drug on our own species. The pharmacodynamics should be well enough worked out that predictions as to probable sites of difficulty can be made, and drug manipulation undertaken with an optimal confidence in the results. Failures will undoubtedly occur since tumor cell types do vary in their propensity for concentrating compounds. For this reason, the results should be evaluated in a second model, in yet another laboratory. It is important to keep in mind the pitfalls of models and ask that well-known question: "compared to what?" Finally, when the time of human testing arrives, the new agent must be pitted against the benchmark of the moment in the same individual.

Kudos to the M. D. Anderson group for shedding some light on the Tc-99m stannous citrate story.

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