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Letter to the Editor

An Example of the Role of the Animal Model in Investigating New Techniques

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THE IMPORTANCE of properly controlled animal studies in investigating clinical techniques before they are applied to the human situation cannot be overemphasized. The premature use of investigational techniques in humans before adequate animal studies have been performed is to be deprecated. The role of humanely conducted animal experimentation, the careful selection of an animal model with due regard to its relevance and direct benefit to clinical problems is emphasized before embarking on studies in humans.

Intra-cerebral tumors are notoriously resistant to treatment with chemotherapy [1]. One of the proposed reasons for this is the presence of an intact, functional blood–brain barrier (BBB) around cerebral tumors preventing the passage of the drugs from plasma to tumor [1]. This barrier, which represents a specialized vasculature in the central nervous system, will under physiological conditions restrict the flow of lipid insoluble, ionized and large molecular weight substances (i.e. MW > 400) from the plasma to the CNS. As a number of cytotoxics used in clinical practice exhibit the above characteristics, it has been suggested that they would be inactive against cerebral tumors due to their inability to cross the BBB and achieve cytotoxic concentrations within the tumor [2, 3].

Assuming that intra-cerebral tumors, as do tumors in other tissues, exhibit a dose–response curve to chemotherapy, and that an intact BBB exists around intra-cerebral tumors, then any technique that would disrupt the BBB would theoretically increase the concentration of intra-cerebral cytotoxic and thereby produce an improved tumor response rate and presumably improve survival of the host.

Since Rapoport [4] described the technique of BBB disruption interest surged in the use of this as an adjunct to the treatment of intra-cerebral tumors in humans. The technique as described by Rapoport involves the infusion of a hyperosmolar agent, e.g. mannitol, into the internal carotid artery. Cosolo *et al.* [5] have shown that the infusion of mannitol (0.25 ml/s/kg for 30 s) into the internal carotid arteries of normal rats to be safe and the cardiovascular changes to be spontaneously reversible. Furthermore, Neuwelt *et al.* [2, 3] and Cosolo *et al.* [6] have demonstrated that in animals BBB disruption produces a many-fold increase in the cytotoxic drug concentration in the hemisphere infused with mannitol. The increase in cytotoxic concentration is also achievable within intra-cerebral tumors of the infused hemisphere.

Prior to its use in humans this technique did not undergo properly controlled and randomized studies in animals. A number of Phase 1 studies in humans have been performed and tumor responses were documented on CT imaging [7]. Neuwelt *et al.* [8] described three cases in which blood–brain barrier disruption was used to treat primary and secondary cerebral tumors. The patients were anesthetized using N_2O_2, thiopental and halothane. After intubation the femoral artery was cannulated and the carotid artery infused with mannitol under CT guidance. In all three cases a response to treatment on CT scan was obtained but whether this was due to the chemotherapy alone or in combination with BBB disruption cannot be determined. Furthermore, whether the survival noted

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using BBB disruption was longer than chemotherapy alone or no therapy cannot be evaluated. The incidence of toxicity reported with this procedure varies, but by taking the average of the reported series, conjunctivitis occurred in 100% of the Bonstelle series, seizures in 20%, brain swelling in 11% and death within 30 days of the procedure in 16%. Neuwelt *et al.* [8] also used the technique in three cases of cerebral lymphoma and surmised that the exact role of the BBB in modulating the response to chemotherapy was unknown. Recently this technique was reported as a useful modality in the treatment of human cerebral lymphomas [9]. However, there have been no randomized double blind studies examining the effect of this treatment on survival in humans.

Cosolo and Christophidis [10] developed an animal model of intra-cerebral tumors and the role of BBB disruption in the treatment of these tumors was investigated. Following Hospital Ethics Committee approval, a controlled randomized study was performed using a readily transplantable osteogenic sarcoma of rats. Animals after stereotactic intra-cerebral inoculation of the osteogenic sarcoma cells were treated with varying doses of intra-venous or intra-carotid methotrexate (a large molecular weight, lipid insoluble, ionized molecule which does not cross an intact BBB) and randomized to BBB disruption or a sham operation. No survival benefit for the groups treated with BBB disruption was noted. In fact, the groups in which the BBB was disrupted did statistically significantly worse.

Although intra-cerebral levels of cytotoxic can be increased following BBB disruption, survival was not improved in this model. This would suggest that it is not the BBB that is important in modulating the response to chemotherapy of cerebral tumors but the type of cell that survives in the CNS.

BBB disruption has been purported to be safe in animals. This animal model demonstrated complications such as intra-cerebral hemorrhage and methotrexate encephalopathy which would correlate with the results of the human studies. It should be noted that in normal rats BBB disruption does not produce complications. This suggests that methotrexate, friable tumor circulation and BBB disruption in combination are toxic. To further complicate matters this technique in humans as described above requires a general anesthetic, intubation, femoral cannulation and carotid angiography under CT guidance. We would thus caution against the use of this technique as an adjunct to the treatment of intra-cerebral tumors.

Thus, the proper use of the animal model allowed proper investigation of a therapeutic modality.

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