SHORT COMMUNICATION: Metrazol enhances brain penetration and therapeutic efficacy of some anticancer agents: implications for brain metastases

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The concurrent administration of Metrazol (60 mg/kg, i.v.) to anaesthetized rats enhances the cerebral penetration of the anticancer agent razoxane. Such an enhancement leads to an increase in the therapeutic efficacy of razoxane against intracerebrally sequestered L1210 leukaemia cells in mice. The combination of Metrazol and melphalan was also examined to see if the concentration of other anticancer agents in CSF could be enhanced.

Introduction

The blood-brain barrier at the cerebrovascular endothelium [17] restricts the passage of polar drugs into the normal brain [2, 4, 15]. In brain tumours the barrier integrity is often lost within the tumour’s centre, but despite this, polar anticancer agents appear unable to reach the infiltrative margins of the tumour in cytotoxic concentrations [10, 19]. This area of the tumour has the highest mitotic activity [9], and thus would be expected to be the most susceptible to anticancer agents.

There are a number of possible factors that could account for this drug distribution problem. Cells at the infiltrative edge appear before tumour-induced neovascularization and are supplied by the capillaries of the adjacent brain [7, 11]. The cerebrovascular permeability of these capillaries is only approximately one half of those in normal brain [11]. Tumour blood flow, and hence drug delivery, is often low [1]. The brain parenchyma surrounding a tumour acts as a diffusion sink [7, 10] and brain tumours as a rule have an efficient perivascular drainage [7].

As a consequence of poor drug access to critical growing regions within intracerebral (i.c.) tumours there has been considerable research into the development of innocuous techniques to increase the cerebrovascular permeability of normally excluded substances of potential use in brain tumour chemotherapy [6, 14–16].

Metrazol, pentylenetetrazol, a CNS stimulant which at high concentrations acts as an analeptic, has been shown to increase the brain penetration of the sparingly water-soluble anticancer agent razoxane in anaesthetized rats by a factor of 2.6 [6]. Quantitative studies with intravascular protein markers have demonstrated that these alterations in cerebrovascular permeability are temporary and reversed within 4 hours [6, 12].

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Experiments were undertaken to assess whether an increase in the cerebral penetration of razoxane would result in an increase in therapeutic efficacy. Pharmacokinetic analysis of the combination of Metrazol + melphalan was also undertaken to ensure that the actions of Metrazol on cerebrovascular permeability were not unique to its combination with razoxane.

**Materials and methods**

L1210 murine leukaemia tumour cells (1 × 10⁵) were injected i.c. into 20 g BDF₁ mice [7]. With the exception of the controls, the animals were injected once daily for 4 days, starting 24 hours after implantation, with either razoxane (100 mg/kg, i.p.), Metrazol (60 mg/kg, i.v.) or Metrazol + razoxane. Animals receiving Metrazol were anaesthetized with Na pentobarbital (30 mg/kg, i.p.). All animals were studied until death occurred.

Melphalan (10 mg/kg in 0.5 per cent CMC saline) was injected i.p. into anaesthetized (30 mg/kg Na pentobarbital) and conscious 200 g Sprague–Dawley rats. Anaesthetized animals were injected with Metrazol (60 mg/kg, i.v.) 2 min later. At intervals after melphalan administration (figure 1) blood and CSF were taken and the animals killed. Blood was withdrawn by cardiac puncture, placed in a heparinized vial, centrifuged (7000 g, 5 min, 4°C), and the plasma kept. CSF was obtained by percutaneous sampling from the cisterna magna [5]. Both were stored in solid CO₂ prior to HPLC analysis.

Samples were analysed by a modification of the method of Chang et al. [3]. Samples of plasma (200 μl) were precipitated by the addition of methanol (400 μl) and placed in a carbon dioxide/methanol freezing mixture. The precipitate was removed by centrifugation at 1000 g for 20 min at 4°C and 50 μl aliquots of the supernatant analysed by HPLC. Twenty-microlitre aliquots of CSF were analysed directly without pretreatment. HPLC was performed on a Waters Associates

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Figure 1. Rat plasma and C.S.F. concentrations of melphalan alone and in combination with Metrazol.