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A Comparison of Blood-Brain Barrier Disruption by Intracarotid Iohexol and Iodixanol in the Rabbit

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A rabbit model was used to compare the effect on the blood-brain barrier of the intracarotid injection of two new contrast media: iohexol, a nonionic monomer, and iodixanol, a nonionic dimer. It was hypothesized that the low osmolality of iodixanol (272 mOsm/kg at 300 mgI/ml) would cause less disruption of the blood-brain barrier than the relatively higher osmolality of iohexol (690 mOsm/kg at 300 mgI/ml). The degree of blood-brain barrier disruption was assessed qualitatively, by observing the degree of cortical staining with Evans' Blue dye, and quantitatively, by calculating the difference in uptake of 99mTc-pertechnetate between injected and noninjected hemispheres. Statistical analysis of the results showed that both iodixanol and iohexol had a significantly greater effect on blood-brain barrier disruption than did isotonic saline ($0.005 > p > .001$), but that the effect of iodixanol was not significantly different from that of iohexol with respect to either Evans' Blue staining ($p > .05$) or pertechnetate uptake ($.75 < p < .90$).

Thus, the low-osmolality iodixanol has no significant advantage over iohexol in terms of blood-brain barrier disruption after experimental carotid angiography.

Direct intracarotid injection of contrast medium is still necessary to image the carotid arteries in a significant number of cases, despite the introduction of digital subtraction angiography after IV contrast injection. Many studies have shown that intracarotid injection of contrast material and other substances can cause a disruption of the blood-brain barrier (BBB), with subsequent leakage from the bloodstream into the brain parenchyma [1–12]. The degree of disruption of the BBB has been shown to depend both on the osmolality of the injected material [2, 4, 6] and on its chemical nature [7, 8]. Previous studies from this laboratory have shown that low-osmolality, tri-iodinated, monomeric, nonionic (ratio 3.0) contrast media cause significantly less disruption of the BBB than high-osmolality, tri-iodinated, monomeric, ionic (ratio 1.5) contrast media after intracarotid injection at equivalent iodine concentrations [9–12].

In the work described here, a previously established rabbit model [12] is used to test the hypothesis that iodixanol—a new, six-iodine, dimeric, nonionic (ratio 6.0) contrast material of low osmolality—will cause less disruption of the BBB than iohexol—a ratio 3.0, nonionic contrast material of approximately two-fold higher osmolality. Both compounds have been developed by Nycomed International, Oslo. Iohexol has been extensively tested and is currently marketed for intravascular and intrathecal use as Omnipaque. Iodixanol contains six iodine atoms on two fully substituted benzene rings (Fig. 1). This compound is highly water-soluble, stable in aqueous solution, and, in preliminary testing, has been shown to be better tolerated than other ratio 3.0, nonionic contrast media (Aulie-Michelet A, personal communication). Since it has twice as many iodine atoms per molecule as ratio 3.0, nonionic compounds, such as iohexol and iopamidol, iodixanol has approximately half the osmolality of these contrast media at equivalent iodine concentrations.

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Materials and Methods

Iohexol and iodixanol were supplied by Nycomed International in 10-ml vials at concentrations of 300 and 320 mgI/ml, respectively. Sterile water was added to each vial of iodixanol to give a final concentration of 300 mgI/ml. Vials containing 10 ml of sterile isotonic (0.9%) saline served as controls. The osmolality of iohexol was determined by the manufacturer to be 690 mOsm/kg at 300 mgI/ml [13], while the osmolality of iodixanol, at an equivalent iodine concentration, was measured at 272 ± 4 mOsm/kg (mean ± SD) from five determinations of freezing point depression using an Advanced Instruments osmometer.

Vials were coded and assigned randomly, and assessment of individual studies was carried out before the code was broken. A total of 10 studies was performed with each of the three test solutions.

Rabbits (IMVS strain, 2.1–3.8 kg) of either sex were anesthetized with a 25% solution of urethane in 0.9% saline, injected into a marginal ear vein at a dose of 7 ml/kg. Each animal was placed supine and a tracheostomy performed. The left common carotid artery was exposed and ligated caudally and a polyethylene catheter (0.5 mm internal diameter) was inserted anterograde into the artery so that the tip rested in the infundibulum of the left internal carotid artery. The left external carotid artery was ligated to ensure that the entire volume of injected test solution was delivered to the cerebral vessels. If the occipital artery branched from the internal carotid artery this was also ligated.

A small region of the skull on the left side was trephined to expose a pial vessel. The test solution was injected manually through the catheter at a rate just sufficient to clear the blood from the pial vessel for a 30-sec injection period. Test solutions were administered at 37°C. Behavioral reactions to the test injections were observed and recorded on an arbitrarily defined scale of severity as follows: no reaction (grade 0); slight reaction (i.e., quivering whiskers) ceasing at end of injection (grade 1); moderate to severe reaction (i.e., very rapid twitching and/or head movements) persisting for several seconds after end of injection (grade 2). The mean injection volumes of the two contrast media were approximately equal and less than half that of saline (Table 1) due, probably, to their higher viscosities.

Immediately after the carotid injection, 99mTc-pertechnetate (100 μCi [3.7 MBq] in approximately 0.2 ml saline) was injected intravenously, followed by 2% Evans' Blue in 0.9% saline at a dose of 3 ml/kg. Thirty minutes after the pertechnetate injection, 1 ml of cardiac blood was removed and the animal was killed immediately by IV anesthetic overdose.

The brain was then removed as quickly as possible and rinsed in 0.9% saline to remove superficial blood and CSF. A subjective assessment of the degree of Evans' Blue staining was made on the basis of a previously established scale [8]. The brain was bisected mid-sagitally and each hemisphere weighed. The pertechnetate activity of the blood sample and of each hemisphere was then counted in a Searle gamma counter using a well attachment.

After correcting for background radiation, the ratio of brain activity (cpm/gm) to blood activity (cpm/ml) was calculated for each hemisphere. The difference between the ratios for each hemisphere was calculated. This value is described as pertechnetate uptake.

Statistical analysis of pertechnetate uptake was carried out using one-way analysis of variance, with orthogonal comparisons between the three treatment groups [14]. Treatment groups were also compared for severity of reaction to test injection and Evans' Blue staining indexes. For these comparisons, the Mann-Whitney U test was employed.

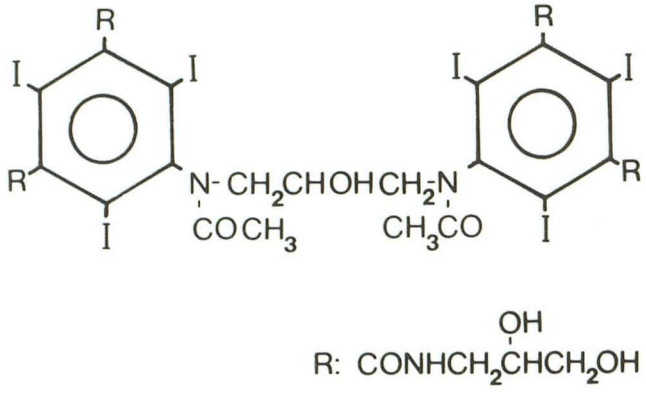

Fig. 1.—The structure of iodixanol.

Results

The results of this study are summarized in Table 1. The mean pertechnetate uptake after iodixanol was less than the mean uptake after iohexol; but the mean uptake after saline injection was considerably lower than after either of the two contrast media. Analysis of variance revealed a significant difference between the three treatment groups ($p < .01$). In orthogonal comparisons between the pooled treatment groups (iohexol and iodixanol) and the control (saline) group, or between the iohexol and iodixanol groups, the pooled treatment groups differed significantly from the saline group ($.005 > p > .001$) but the iodixanol group did not differ significantly from the iohexol group ($.75 < p < .90$).

In the saline group, only one animal showed Evans' Blue staining, which was +. In the iohexol and iodixanol groups, animals displayed 0, +, and ++ staining; but there were fewer cases of ++ staining in the iodixanol group than in the iohexol group. There was no example of +++ staining in any of the

TABLE 1: A Summary of Pertechnetate Uptake, Staining Index, and Behavioral Reaction After Intracarotid Injection of Saline, Iohexol, or Iodixanol

	Pertechnetate Uptake	Staining Index				Reaction			Injection Volume (ml)
	(mean ± SD, $n = 10$)	0	+	++	+++	0	1	2	(mean ± SD, $n = 10$)
Saline	0.0044 ± 0.0098	9	1	—	—	10	—	—	8.2 ± 1.1
Iohexol	0.0246 ± 0.0195	3	3	4	—	1	5	4	3.3 ± 1.2
Iodixanol	0.0167 ± 0.0092	4	5	1	—	—	8	2	3.0 ± 0.9

groups. No significant difference could be detected between the staining indexes of the iohexol and iodixanol groups if they were analyzed with the nonparametric Mann-Whitney U test ($p > .05$). In both the iodixanol and iohexol groups there appeared to be a positive relationship between staining index and pertechnetate uptake, with a higher staining index usually correlating with higher pertechnetate uptake (Fig. 2A).

None of the animals showed a behavioral reaction after injection of saline. In the iodixanol and iohexol groups, however, all but one animal showed reactions, ranging from slight to severe. The iodixanol group displayed fewer moderate to severe (grade 2) reactions than the iohexol group but there was no significant difference between the reactions of the two contrast media groups when analyzed with the Mann-Whitney U test ($p > .05$). There was no obvious relationship between the grade of reaction to injection and pertechnetate uptake in the two contrast media groups (Fig. 2B).

Discussion

The osmolality of contrast material is known to play a major role in the disruption of the BBB after carotid angiography [2, 4]. Consequently, the use of contrast material with reduced osmolality would be expected to result in a decrease in BBB disruption. In this study, the disruption of the BBB after intracarotid injection of iodixanol—a new, approximately isotonic, nonionic, ratio 6.0 contrast medium—or iohexol—a currently marketed nonionic, ratio 3.0 contrast medium of somewhat higher osmolality—was compared using an established rabbit model [12]. The degree of disruption of the BBB was assessed grossly by observing the amount of leakage of Evans' Blue dye from cortical blood vessels, and, more sensitively, by calculating the difference in the uptake into the brain parenchyma of radiolabeled pertechnetate between injected and noninjected hemispheres.

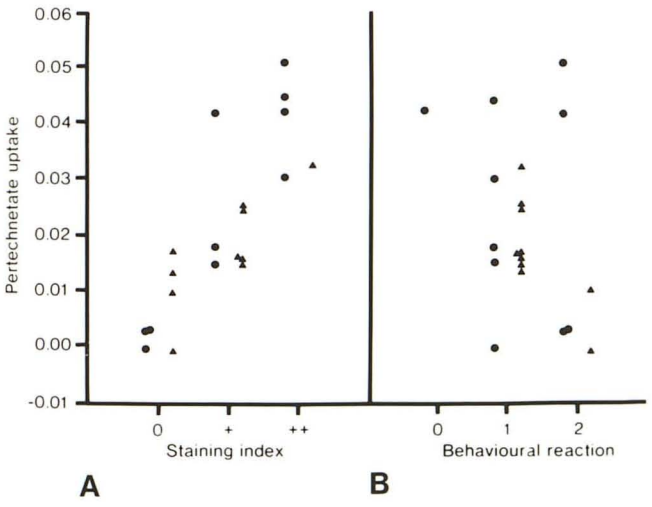

Fig. 2.—*A*, Evans' Blue staining index.
***B*, Grade of behavioral reaction to the injection plotted against degree of BBB disruption as measured by amount of pertechnetate uptake for each study with iohexol (●) and iodixanol (▲). The staining indexes and grades of behavioral reaction are defined in the text.**

Both iodixanol and iohexol were found to have a significantly greater disruptive effect on the BBB than did saline. Both pertechnetate uptake and the degree of staining with Evans' Blue were moderately lower after iodixanol than after iohexol; however, the decrease is not statistically significant at the 5% probability level. It should be noted, however, that under similar experimental conditions, iohexol, and by extrapolation iodixanol, has significantly less effect on the BBB than does methylglucamine iothalamate, a conventional ionic contrast material [11]. There was also no significant difference between the behavioral reactions to the two contrast materials in this study. The significance of these reactions is unclear, but they do not appear to be correlated with BBB disruption.

A recent study in this laboratory compared the effects on the rabbit BBB of iotrol—a six-iodine, dimeric, nonionic contrast medium analogous to iodixanol—with iohexol, iopromide, and methylglucamine iothalamate [15]. The results obtained were similar to those of the present study, with iotrol appearing to have less effect on the BBB than iohexol but with the difference not being statistically significant. Iotrol was found not to differ significantly from saline; however, in this earlier study, contrast media were tested at an iodine concentration of 200 mgI/ml while in the present study they were tested at a concentration of 300 mgI/ml. In both these studies and in others carried out in this laboratory [9–12, 15], measurements of BBB disruption using pertechnetate uptake, Evans' Blue staining, or CT contrast enhancement have consistently ranked tested substances as follows: saline < nonionic dimers < nonionic monomers < ionic monomers. Although the probability of there being no significant difference is in some cases relatively high, as for example with iotrol/iohexol and iodixanol/iohexol, the reproducibility of the ranking together with the combined probabilities suggests that there may be some validity in the ranking.

The osmotic threshold for opening of the rabbit BBB to Evans' Blue-albumin has been shown to be approximately 1200 mOsm using a model very similar to that of the present study [2]. In this study, both iohexol and iodixanol were used at osmolalities well below the apparent osmotic threshold; yet, at least 60% of the animals in both groups showed some degree of Evans' Blue staining. This finding, together with the finding that there was no significant difference between iohexol and iodixanol with respect either to pertechnetate uptake or to Evans' Blue staining index, suggests that the effects on the BBB observed here are related to some factor other than osmolality. Intravascular injection of contrast material produces other adverse effects that are either dependent on osmolality (e.g., vasodilatation, hypervolemia, and erythrocyte rigidity) or apparently independent of it (e.g., "allergic" reactions) [16]. Although statistically the low osmolality of iodixanol has not proven to have any advantage over the higher osmolality of iohexol with respect to BBB disruption in this study, further testing will reveal whether iodixanol produces fewer of the other, osmolality-related, adverse effects than do iohexol and other, similar, ratio 3.0, nonionic contrast media.

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