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The Effect of Contrast Medium Viscosity on the Blood-Brain Barrier After Intracarotid Injection in the Rabbit

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This study was undertaken to investigate the role of contrast medium viscosity in blood-brain barrier disruption after carotid angiography. Test solutions were injected into the carotid arteries of rabbits, and the degree of disruption was assessed by using ^{99m}Tc-pertechnetate and Evans blue as quantitative and qualitative markers, respectively. The seven test solutions consisted of basic solutions of physiological saline, iopromide, or methylglucamine iothalamate plus solutions derived from these by the addition of sufficient gelatin to augment their viscosities considerably. The solutions were injected over a 30-sec period, resulting in doses that varied inversely with viscosity. One of the high-viscosity solutions was also injected as a fixed dose, equal to the mean injection volume of its low-viscosity counterpart, without regard to the time used.

Statistical comparison between the effects of the solutions showed that, under the conditions of the study, contrast medium viscosity, either by itself or as a consequence of its association with hyperosmolality, has no significant effect on the blood-brain barrier. However, under conditions of constant injection volume, higher viscosity solutions may require increased injection times, and this may lead to increased disruption of the blood-brain barrier.

Numerous experimental and human studies have shown that carotid angiography may result in a temporary disruption of the blood-brain barrier (BBB) [1–13]. The hyperosmolality of the contrast medium has been implicated as a major factor in this disruption [1, 4–7, 9–12], while contrast medium chemotoxicity also appears to play a part [2, 4, 14].

Contrast media, at the concentrations normally used in carotid angiography, have viscosities higher than that of blood. The nonionic monomers, such as iohexol, iopamidol, and iopromide, and the ionic dimer ioxaglate have viscosities that are higher than those of the ionic monomers [15]. The nonionic dimers currently being investigated, such as iotrolan, are more viscous than any of the contrast media commercially available [10]. It has been shown that after the intracarotid injection of solutions of higher viscosity than blood there is a transient decrease in downstream flow [16]. This decrease is more pronounced with higher viscosity solutions and appears to correspond to the period when they are passing through the microcirculation. This increased transit time would be expected to expose the cerebrovascular endothelium to these solutions for an increased time, since contrast media usually are hyperosmotic. Hyperosmolality and duration of contact with the endothelium are factors that are closely related to the production of BBB disruption [17].

Two previous studies from this laboratory have investigated the relationship of contrast medium viscosity and BBB disruption [8, 13]. The studies were performed with a canine and a rabbit model and used contrast enhancement, as measured by CT, and extravasation of radiolabeled pertechnetate, respectively, as markers of BBB disruption. Both studies used methylglucamine iothalamate (MGI) injected at 23°C or 37°C, at which temperatures its viscosity measures 5.0 or 3.2, respectively [8, 13]. In neither study did viscosity have a significant effect.

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In our present study, a blind comparison was made of the disruptive effect of the intracarotid injection of various test solutions on the rabbit BBB by using ^{99m}Tc-pertechnetate and Evans blue as markers of disruption. The test solutions fell into three classes according to whether they were based on physiological saline, nonionic contrast medium (iopromide), or an ionic contrast medium (MGI). Within the three classes, test solutions consisted of either the basic solutions or the same solutions to which gelatin had been added. Sufficient gelatin was added to produce a large increase in viscosity but only a small increase in osmolality. Thus, within classes, the major difference between test solutions was in their viscosities, while their chemical compositions and osmolalities differed only slightly.

Materials and Methods

The seven test solutions were supplied in sterile 20-ml vials by Schering AG, Berlin. Their viscosities and osmolalities were measured in this laboratory with a U-tube viscometer and a vapor pressure osmometer, respectively, and are shown in Table 1, together with their compositions. The vials were coded and assigned randomly, with assessment of individual studies before the code was broken. Ten studies were performed with each of the seven test solutions.

Rabbits (IMVS strain, 1.7–4.3 kg) of either gender were anesthetized with a 25% solution of urethane in physiological saline, which was injected into a marginal ear vein at a dose of 7 ml/kg. The level of anesthesia was such that the forelimb withdrawal response was abolished. Each animal was placed supine and a tracheostomy was 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 until its tip lay at the origin 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 ligated also.

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: grade 0 = no reaction; grade 1 = slight reaction (e.g., quivering whiskers, which ceased at the end of injection); and grade 2 = moderate to severe reaction (e.g., very rapid twitching and/or head movements, persisting for several seconds after the injection ended). The mean injection volumes of the seven test solutions are shown in Table 2; the most viscous solutions had the lowest mean injection volumes.

Immediately after the carotid injection, ^{99m}Tc-pertechnetate (100 μCi [3.7 MBq] in approximately 0.2 ml of saline) was injected IV,

TABLE 1: Composition of the Test Solutions and Their Viscosities and Osmolalities

Group No.	Composition	Viscosity[a]	Osmolality (mosm/kg)[b]
1	Physiological saline	1.0	290
2	Physiological saline + 150 mg gelatin/ml	8.4	390
3	Physiological saline + 200 mg gelatin/ml	18.4	430
4	Iopromide (300 mg I/ml)	5.3	610
5	Iopromide (300 mg I/ml) + 50 mg gelatin/ml	14.0	690
6	Methylglucamine iothalamate (280 mg I/ml)	4.4	1490
7 and 8	Methylglucamine iothalamate (280 mg I/ml) + 60 mg gelatin/ml[c]	12.5	1620

[a] At 37°C, relative to water.
[b] At 37°C.
[c] Group 7 received a variable dose injected over a fixed period, while group 8 received a fixed dose injected over a variable period (see text).

TABLE 2: Summary of Pertechnetate Uptake, Staining Index, and Behavioral Reaction after Intracarotid Injection of Test Solutions

Group No.	Pertechnetate Uptake ($\times 10^3$)[a]	Staining Index				Reaction Grade			Injection Volume (ml)[a]
		0	+	++	+++	0	1	2	
1	3.4 ± 8.8	9	1	0	0	10	0	0	8.6 ± 1.3
2	1.5 ± 5.7	8	2	0	0	10	0	0	3.8 ± 0.9
3	8.0 ± 9.3	7	3	0	0	10	0	0	2.5 ± 0.6
4	11.3 ± 14.3	7	0	3	0	1	4	5	3.6 ± 0.9
5	12.9 ± 17.5	7	1	2	0	3	2	5	2.5 ± 0.4
6	25.8 ± 21.6	3	1	3	3	10	0	0	4.2 ± 1.1
7	30.2 ± 19.6	1	2	6	1	10	0	0	2.6 ± 0.7
8	44.8 ± 27.5	0	2	3	5	10	0	0	4.2[b]

Note.—Staining index and reaction grade values are arbitrary. Reaction grades 0, 1, and 2 represent no reaction, slight reaction, and moderate to severe reaction, respectively.
[a] Mean ± SD (n = 10).
[b] All injected with same volume.

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 [4]. The brain was bisected midsagittally and each hemisphere weighed. The pertechnetate activity of the blood sample and of each hemisphere was then counted in a Searle gamma counter with a well attachment.

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

A further group of 10 animals was studied (group 8), in which each animal was injected with a fixed volume (4.2 ml) of high-viscosity MGI for whatever time was necessary to inject the dose, while keeping the exposed pial vessel clear of blood. The test solution was identical to that in group 7, and the mean injection time was 52.2 ± 10.6 sec (mean ± SD). In all other respects, the animals in this group were treated in the same way as those in the other seven groups. The fixed injection volume was chosen because it was also the mean injection volume of the low-viscosity MGI group (group 6) (Table 2). This permitted a comparison of the effects of the injection of identical doses of low- and high-viscosity MGI; that is, between groups 6 and 8.

Statistical analysis of the results was carried out by using the SPSS statistical analysis software package.* Differences in pertechnetate uptake were analyzed by using one-way analysis of variance together with the Student-Newman-Keuls range test. Differences in the Evans blue staining index or degree of behavioral reaction were analyzed by using the Mann-Whitney U test.

Results

The results are summarized in Table 2, which shows the mean pertechnetate uptake, the Evans blue staining indexes, and the degree of behavioral reaction for each of the eight treatment groups. The mean pertechnetate uptake ranked the groups in the same order as did osmolality, except for the medium-viscosity saline group (group 2), which had the lowest pertechnetate uptake. The degree of Evans blue staining also generally increased with increasing osmolality. The only behavioral reactions observed during or shortly after treatment occurred in the two iopromide groups (groups 4 and 5).

The statistical comparisons between the treatment groups on the basis of pertechnetate uptake and Evans blue staining are shown in Table 3. Although the high-viscosity saline group (group 3) had a higher mean pertechnetate uptake than did the normal saline group (group 1), which in turn had a higher uptake than the medium-viscosity group (group 2) (Table 2), analysis of variance showed that there was no significant difference between the three saline groups. Similarly, although there was a slight increase in the number of animals showing Evans blue staining with increasing viscosity (Table 2), this increase was not significant (Table 3).

The high-viscosity iopromide group (group 5) had a higher mean pertechnetate uptake than did the normal (low-viscosity) iopromide group (group 4) (Table 2); this difference was not significant (Table 3). The difference in the degree of Evans blue staining likewise was not significant. The behavioral reactions observed only in these two groups were not significantly different ($p > .6$). Neither of the two iopromide groups differed significantly from any of the three saline groups, either in pertechnetate uptake or in Evans blue staining.

The high-viscosity MGI group (group 7) had a higher pertechnetate uptake than did the normal (low-viscosity) MGI group (group 6) (Table 2); however, this difference was not significant, and neither was the difference in the degree of Evans blue staining (Table 3). The normal MGI group (group 6), however, was significantly different from the normal- and medium-viscosity saline groups (groups 1 and 2), while the high-viscosity MGI group (group 7) was significantly different from all three saline groups. These differences were also significant in terms of the degree of Evans blue staining. All other test groups (with the exception of group 8) did not differ significantly from the two MGI groups (groups 6 and 7) on the basis of pertechnetate uptake. On the basis of Evans blue staining, however, four of these cross-comparisons (normal

* SPSS Inc., Chicago, IL.

TABLE 3: Statistical Comparisons Between the Effects of Test Solutions on the Blood-Brain Barrier on the Basis of Pertechnetate Uptake or Evans Blue Staining

Group No., Composition	Pertechnetate Uptake/Evans Blue Staining						
	1, Saline	2, Saline	3, Saline	4, Iopromide	5, Iopromide	6, MGI	7, MGI
1, Saline	–	–	–	–	–	–	–
2, Saline + 150 mg gelatin/ml	NS/NS	–	–	–	–	–	–
3, Saline + 200 mg gelatin/ml	NS/NS	NS/NS	–	–	–	–	–
4, Iopromide	NS/NS	NS/NS	NS/NS	–	–	–	–
5, Iopromide + 50 mg gelatin/ml	NS/NS	NS/NS	NS/NS	NS/NS	–	–	–
6, MGI	S/S	S/S	NS/S	NS/NS	NS/S	–	–
7, MGI + 60 mg gelatin/ml[a]	S/S	S/S	S/S	NS/S	NS/S	NS/NS	–
8, MGI + 60 mg gelatin/ml[a]	S/S	S/S	S/S	S/S	S/S	S/NS	NS/NS

Note.—MGI = methylglucamine iothalamate; NS = not significant; S = significant ($p < .05$) (analysis of variance, Student-Newman-Keuls test/Mann-Whitney U test).

[a] In group 7, a variable dose was injected over a fixed period; in group 8, a fixed dose was injected over a variable period.

MGI vs high-viscosity saline and high-viscosity iopromide, and high-viscosity MGI vs normal iopromide and high-viscosity iopromide) did prove to be significantly different. These discrepancies probably reflect the qualitative, subjective nature of the Evans blue technique and its consequently reduced sensitivity in comparison with the pertechnetate technique.

On the basis of pertechnetate uptake, group 8, in which animals were injected with high-viscosity MGI irrespective of the time taken, was significantly different from all other groups except the group receiving high-viscosity MGI injected in 30 sec (group 7) (Table 3). This was also found to be true for Evans blue staining, except that, in addition, the normal MGI group (group 6) did not differ significantly from group 8.

Discussion

The contrast media currently in use in carotid angiography have osmolalities and viscosities higher than that of blood. Osmolality has been shown to be a major factor in the disruption of the BBB observed after experimental carotid angiography [1, 4–7, 9–12], while the contribution of viscosity has been investigated in two previous studies from our laboratory [8, 13]. In these two studies, both of which revealed no significant effect of viscosity, MGI was injected into the carotid artery at a temperature of either 23°C or 37°C, with a consequent difference in its viscosity. This difference was, however, not great, the viscosity being 3.2 and 5.0 at 37°C and 23°C, respectively, expressed relative to water at 20°C. In our present study, the disruptive effects of the intracarotid injection of solutions of physiological saline, iopromide, or MGI were compared with those brought about by injection of the same solutions to which gelatin had been added. Sufficient gelatin was added to produce viscosity differences much greater than those in the previous two studies, while the osmolalities of the solutions increased by a maximum of 140 mosm/kg (Table 1). This method eliminates the complicating factor of the warming and subsequent decrease in viscosity of solutions injected at temperatures below that of blood. One of the previous studies was carried out in a canine model, and CT was used to measure the amount of contrast medium iodine penetration into the brain as an indication of BBB disruption [8]. It has been shown that this technique is not as sensitive as that used in our present study and another previous study, in which ^{99m}Tc-pertechnetate was used as a marker of BBB disruption in an established rabbit model [9, 12].

Statistical analysis of the present results revealed no significant differences between any of the three saline test groups with respect to their disruptive effects on the BBB as measured by pertechnetate uptake. This means that the displacement of the blood in cerebral vessels by a highly viscous, physiologically inert solution causes no more damage to the BBB than does displacement of the blood by a low-viscosity inert solution, despite the fact that the highly viscous solution is likely to persist longer before being washed out. With the 30-sec injection regime, therefore, it appears that the intrinsic property of high-viscosity solutions of persisting in cerebral vessels, thereby causing a prolonged exclusion of blood, was not by itself a significant factor in BBB disruption. Similarly, the high-viscosity iopromide and MGI solutions were no more damaging to the BBB than were their low-viscosity counterparts. The osmolalities of the iopromide and MGI solutions were, respectively, approximately two and five times higher than that of physiological saline (Table 1), which suggests that an increase in viscosity did not potentiate the effect of these hyperosmotic solutions on the BBB. This in turn suggests that the combination of hyperosmolality and increased contact time between the highly viscous contrast media and the cerebrovascular endothelium was not sufficient to cause a significant increase in the breakdown of the BBB. Furthermore, our present findings are consistent with general observations from previous work [5–7, 9–12] that low-osmolality contrast media are less deleterious to the BBB than are high-osmolality agents.

In groups 1 to 7, in which a 30-sec injection period was used, the injection volume varied inversely with solution viscosity (Tables 1 and 2). To compare the effects of identical injection volumes of solutions of differing viscosities, the same volume of high-viscosity MGI was injected into a further group (group 8), irrespective of the time used to inject it. Since the viscosity was much greater, the injection time varied between 40 and 70 sec (mean, 52 sec) as compared with the standard 30-sec injection time in group 6. Statistical analysis indicates that the high-viscosity, increased-duration injection regime of group 8 was significantly more damaging to the BBB than was the lower-viscosity, 30-sec regime of group 6. It is not clear what proportion of this increase is attributable to viscosity alone and what proportion is attributable to the increased injection time, although the two factors are to a certain extent interrelated.

If a similar, fixed-dose, increased-duration injection regime had been carried out in the high-viscosity saline and iopromide groups, it is probable that BBB damage would not have differed significantly from that of their low-viscosity counterparts, in contradistinction to the difference observed between groups 6 and 8 (Table 3). This is because the high-viscosity saline and iopromide groups have osmolalities about 25% and 40%, respectively, that of the high-viscosity MGI group (Table 1); Rapoport et al. [17] have shown that the hyperosmotic BBB opening has a threshold that seems to be dependent on the product of injection duration and injectant osmolality. In group 8, the combination of high viscosity, high osmolality, and long injection time was evidently sufficient to cause a significant increase in BBB disruption. However, extrapolation from the findings of Rapoport et al. suggests that even moderately hyperosmotic contrast media such as iopromide, iohexol, and iopamidol are likely to cause BBB disruption if injected for long enough. It should also be pointed out that the significant increase in BBB breakdown seen in group 8 was brought about by a contrast medium with a viscosity approximately twice, and with an osmolality more than twice, that of current nonionic angiographic contrast media. In addition, in our model this medium totally displaced the blood in the pial (and presumably cerebral) vasculature for a mean of 52 sec, so that the conditions used here far exceed those used clinically.

The behavioral reactions observed in this study during injection of iopromide have been observed by us in previous studies, where low-osmolality, nonionic media such as iohexol, iotrolan, and iodixanol produced similar reactions [10, 11]. As in this study, reactions to ionic solutions such as saline and MGI were not observed in previous studies either [10, 11]. Whatever the mechanism for these reactions, it appears to be related neither to high viscosity nor to hyperosmolality, since, in this study, injection of high-viscosity, high-osmolality solutions of MGI produced no observable reactions; neither was there any significant difference between the severity of the reactions produced by iopromide solutions of low or high viscosity. These reactions are currently the subject of investigation in our laboratory.

It can be concluded that, under the conditions of this study, contrast medium viscosity, either by itself or in combination with hyperosmolality, has no significant effect on the BBB. However, increased disruption of the BBB may result from increased injection times, which may be necessary to inject equivalent doses of higher viscosity contrast media.

REFERENCES

1. Rapoport SI, Thompson HK, Bidinger JM. Equi-osmolal opening of the blood-brain barrier in the rabbit by different contrast media. *Acta Radiol [Diagn]* (Stockh) **1974**;15:21–32
2. Aulie A. Effect of iohexol, metrizamide and ioxaglate on the blood-brain barrier. *Acta Radiol [Suppl]* (Stockh) **1980**;362:13–16
3. Sage MR, Drayer BP, Dubois PJ, Heinz ER, Osborne DR. Increased permeability of the blood-brain barrier after carotid Renografin-76. *AJNR* **1981**;2:272–274
4. Sage MR, Wilcox J, Evill CA, Benness GT. Comparison and evaluation of osmotic blood-brain barrier disruption following intracarotid mannitol and methylglucamine iothalamate. *Invest Radiol* **1982**;17:276–281
5. Sage MR, Wilcox J, Evill CA, Benness GT. Comparison of blood-brain barrier disruption following intracarotid metrizamide and methylglucamine iothalamate (Conray 280). *Australas Radiol* **1982**;26:225–228
6. Sage MR, Wilcox J, Evill CA, Benness GT. Comparison of blood-brain barrier disruption by intracarotid iopamidol and methylglucamine iothalamate. *AJNR* **1983**;4:893–895
7. Sage MR, Wilcox J, Evill CA. The effects of intracarotid contrast media on the blood-brain barrier: iohexol vs methylglucamine iothalamate. *Australas Radiol* **1984**;28:6–8
8. Wilcox J, Sage MR. Is viscosity important in the production of blood-brain barrier disruption by intracarotid contrast media? *Neuroradiology* **1984**;26:511–512
9. Wilcox J, Sage MR. Effects of intracarotid ioxaglate on the normal blood-brain barrier. A comparison of two animal models. *Neuroradiology* **1985**;27:342–345
10. Wilcox J, Evill CA, Sage MR. Effects of intracarotid ionic and non-ionic contrast material on the blood-brain barrier in a rabbit model. *Neuroradiology* **1986**;28:271–274
11. Wilcox J, Wilson AJ, Evill CA, Sage MR. A comparison of blood-brain barrier disruption by intracarotid iohexol and iodixanol in the rabbit. *AJNR* **1987**;8:769–772
12. Wycherley AG, Wilcox J, Sage MR. Comparison of blood-brain barrier disruption in the rabbit following intracarotid iopamidol and methylglucamine iothalamate. *Australas Radiol* **1984**;28:294–296
13. Wycherley AG, Wilcox J, Sage MR, Evill CA. Contrast media viscosity: a contributing factor in blood-brain barrier damage following intracarotid contrast injection? *Australas Radiol* **1986**;30:96–98
14. Grainger RG. Osmolality of intravascular radiological contrast media. *Br J Radiol* **1980**;53:739–746
15. Fischer HW. Catalog of intravascular contrast media. *Radiology* **1986**;159:561–563
16. Morris TW, Kern MA, Katzberg RW. The effects of media viscosity on hemodynamics in selective arteriography. *Invest Radiol* **1982**;17:70–76
17. Rapoport SI, Fredericks WR, Ohno K, Pettigrew KD. Quantitative aspects of reversible osmotic opening of the blood-brain barrier. *Am J Physiol* **1980**;238:421–431