Comparison of the Effects of Ioversol and Other Contrast Media on the Blood-Brain Barrier

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Little information is available concerning the neurotoxicity of ioversol injected in animals. The effects of ioversol and other contrast media as well as vehicle control on the blood-brain barrier (BBB) were examined by injection into the left carotid artery of Mongolian gerbils 1 min prior to the i.v. injection of [8-¹⁴C]dopamine. The animals were sacrificed 1 min after the i.v. injection to prepare the frontal brain sections following the stereotaxic atlas. No uptake of the labeled substance was observed when the vehicle control and ioversol were used, suggesting that exposure to ioversol does not damage the BBB. Both meglumine sodium amidotrizoate and iohexol caused damage to the BBB, as shown in the cerebral cortex, brain stem, and hippocampus sections in the left hemisphere. Iopamidol induced a much less but significant effect. Our results suggest that ioversol is an useful contrast medium.

Keywords ioversol; iodine contrast medium; Mongolian gerbil; blood-brain barrier

The high-osmolality water-soluble contrast media in global use are all derivatives of triiodinated benzoic acid. In the quantities and concentrations required, their use imposes a considerable osmotic load, ranging from 1500 to 2200 mOsm/kg H₂O, compared with serum osmolality of approximately 300 mOsm/kg H₂O. This could cause a variety of physiological perturbations.

Ioversol¹⁾ is a new ratio 3 non-ionic contrast medium which is designed to effectively reduce the number of particles in solution, at any given iodine concentration, to one particle per three iodine atoms, as shown in Fig. 1. Furthermore, its hydrophilicity (octanol/water partition coefficient, $K_{o/w}=4 \times 10^{-4}$) is higher than that of iopamidol ($K_{o/w}=19 \times 10^{-4}$) and iohexol ($K_{o/w}=19 \times 10^{-4}$).²⁾

Based on favorable acute toxicity profiles in animals, relative to conventional ionic and the newer non-ionic contrast media, ioversol is expected to be comparable to, or safer, than the other contrast media currently available.¹⁾ Ralston and Robbins reported³⁾ that opening of the blood-brain barrier (BBB) was evaluated by bilateral intracarotid injection of Tc-99m pertechnetate 1 min after test agent or normal saline (control substance) administration and subsequent calculation of Tc-99m blood/brain retention index 1 min later. Their results show that Tc-99m pertechnetate brain/blood ratios were comparable among all test/control substances. In their studies, not only non-ionic agents such as ioversol, iopamidol, and iohexol but ionic agents such as amidotrizoate (MD50®) and mannitol were examined. These results suggest that their experimental conditions might not have been able to distinguish the differences in damage to the BBB between the control and test substances or among the substances.

In this study, two new ideas were tested: 1) semi-microautoradiographic expression of [8-¹⁴C]dopamine uptake was used for evaluation of damage to the BBB, based on no uptake of dopamine into normal brain⁴⁾; 2) the left intracarotid injection to Mongolian gerbils was used for administration of ioversol, the other test agents and normal saline, to obtain a clear picture concerning the sites and extent of damage to the BBB on the left and

right semispherical brains.

MATERIALS AND METHODS

Materials [14C]Dopamine (3,4-[8-¹⁴C]dihydroxyphenethylamine, ARC-279), purchased from Muromachi Chemicals Co., Ltd., (Tokyo, Japan) was used for the present study. The specific radioactivity of the labeled compound was 2.04 GBq/mmol (55 mCi/mmol), and the radiochemical purity was 99%. Ioversol (Optiray 320; lot No. S86134) and meglumine sodium amidotrizoate (Vascovue 76; lot No. 802065) were supplied by Mallinckrodt Medical Co., Ltd., (Tokyo, Japan). Iopamidol (Iopamiron 370; lot No. 11010) was purchased from Nihon Schering K. K. (Osaka, Japan). Iohexol (Omnipaque 350; lot No. G024) was from Daiichi Pharmaceutical Co., Ltd., (Tokyo, Japan). The structure of ioversol is shown in Fig. 1.

Animals Five-week-old Mongolian gerbils were purchased from Sankyo Labo Service Co., Ltd. (Tokyo, Japan) and acclimated to our study conditions in the animal room for a week. The room temperature was $22 \pm 1^\circ\text{C}$ and the humidity $60 \pm 10\%$. The animals were fed laboratory food (CE-2, Clea Japan, Inc., Tokyo, Japan) and fresh tap water *ad libitum*.

Test Substances All of the test substances were dissolved in sterile water for injection, respectively, to provide 32% I w/v. Two ml of each injection solution was administered within a period of 1 min into the left carotid of Mongolian gerbils under anesthesia. [14C]Dopamine (1.85 MBq/kg body weight in the first experiment and 3.7 MBq/kg body weight in the second experiment) was injected into the caudal vein 1 min after dosing with the

Fig. 1. Structure of Ioversol

test drug. One min after the administration of [^{14}C]dopamine, the animals were decapitated and the brain was immediately removed, frozen, and sliced with a large-type sliding microtome (Leitz 1400 type). The resultant thin sections were vacuum-freeze-dried and exposed to an Imaging Plate (Fuji Photo Film Co., Ltd., Tokyo, Japan) for an optimum period for image processing with a Bioimaging analyzer (BAS 2000, Fuji Photo Film Co., Ltd., Tokyo, Japan).⁵⁾ The plate is a flexible image sensor in which bunches of tiny crystals (grain size: about $5\text{ }\mu\text{m}$), composed of photo-stimulable phosphor of barium fluorobromide and trace amounts of bivalent europium, formulated as BaFBr:Eu^{2+} , as a luminescence center, are uniformly coated at a thickness of $150\text{--}300\text{ }\mu\text{m}$ on a polyester support film. Exposure of samples to the Imaging Plate is performed in a manner similar to that in handling photo-film. An exposed plate is scanned with a He-Ne laser beam of red light (633 nm) while the plate is being conveyed with high accuracy in a phosphor reader. Depending on the purpose, the reading density is selected

from 5 to 20 pixels/mm. The reading sensitivity and sensitivity range can also be selected according to the purpose. A bluish purple (400 nm) photostimulated-luminescence (PSL), released upon laser excitation, is collected through the light collection guide to the photomultiplier tube, and converted there to analog electric signals in chronological order. Subsequently, these are converted to digital signals of 8 to 12 bits, again depending on the intended purpose. Image analysis and data processing are done on a CRT screen.

RESULTS

Figure 2 shows the effects of ioversol and other test agents on the left semispherical brain, as compared to the right semispherical brain which received no test agents. The frontal section pictures numbered in the vertical line were arranged from the anterior position to the posterior position of the bisemispherical brain. The red colored areas indicate a high radioactivity accumulation in each

Fig. 2. Comparison of Characteristic Localization of Photodensity over Frontal Sections of the Brain

One min before i.v. administration of [^{14}C]dopamine, three non-ionic contrast media, ioversol (M), iopamidol (I), and iohexol (O), and one ionic medium, meglumine sodium amidotrizoate (V), were injected, respectively, into the carotid artery of Mongolian gerbils. The numbers on the left refer to 6 different phases according to reference 9.

Fig. 3. Effect of Ioversol on Localization of Photodensity over Frontal Sections of the Brain

One min before i.v. administration of [^{14}C]dopamine, non-ionic contrast media, ioversol, was injected into the carotid artery of Mongolian gerbils. The numbers on the left refer to 3 different phases according to reference 9. The numbers on the bottom refer to animal numbers.

TABLE I. Visual Observation of Autoradiographic Brain Image of Mongolian Gerbils

Region	C		M		I		V		O	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Cerebral cortex	+	+	+	+	+	+	++	+	++++	+
Caudatus nucleus	+	+	+	+	+	+	+	+	+	+
Corpus callosum	±	±	±	±	±	±	±	±	±	±
Seotum	+	+	+	+	+	+	+	+	+	+
Brain stem	++	++	+	+	+	+	++	+	++++	+
Hippocampus	+	+	+	+	+	+	++++	+	++++	+
Ventricle	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++

C, control, saline; M, ioversol; I, iopamidol; V, meglumine sodium amidotrizoate; O, iohexol. Radioactivity: ±, hardly detectable; +, very low; ++, low; +++, moderately high; +++++, high; ++++++, very high.

TABLE II. Relative Intensity $[(\text{PSL} - \text{BG})/\text{S}]^{(a)}$ in the Left Semispherical Brain Section versus the Right Semispherical Brain Section

Region	Test agents				
	C	M	I	V	O
			Ratio		
Cerebral cortex	1.04	1.11	0.96	1.48	2.68
Hippocampus	1.06	0.95	0.88	3.75	4.51
Brain stem	1.01	0.88	0.95	0.97	1.58

^a Relative intensity of the radioactivity $(\text{PSL} - \text{BG})/\text{mm}^2$. C, control, saline; M, ioversol; I, iopamidol; V, meglumine sodium amidotrizoate; O, iohexol.

bisemispherical section. In the physiological control (C), the sites and intensity of the red colored portions on the left and right sides did not differ from each other. The red colored areas show the cross section of blood vessels in the brain section. The pictures of the control animal brain (C) revealed no damage to the BBB following the administration of [^{14}C]dopamine into parenchymatous brain cells. Similar results were obtained for animals

treated with ioversol and iopamidol, indicating that these two compounds also did not cause marked damage to the BBB. Contrary to these two agents, both meglumine sodium amidotrizoate (V) and iohexol (O) caused considerable damage to the BBB. The picture of meglumine sodium amidotrizoate (V) shows relatively definitive uptake of ^{14}C -radioactivity into the hippocampus regions of the left semispherical brain sections of No. 3 and 4. The damage induced by iohexol was much more severe than that by meglumine sodium amidotrizoate (V). Not only hippocampus but also cerebral cortex suffered extensive damage when iohexol was administered to the brain blood vessel. The extent of damage caused by ioversol and other contrast media to the BBB is summarized in Table I. Several zones in the frontal brain section were compared using six optical semiquantitative gradations, *i.e.*, ±, +, ++, +++, and +++++. It is seen that the cerebral cortex, hippocampus, and brain stem were the main critical areas reflecting the damage of the BBB. Therefore, a quantitative analysis of the photo-stimulated luminescence, corresponding to radioactivity,

Fig. 4. Effect of Iopamidol on Localization of Photodensity over Frontal Sections of the Brain

One min before i.v. administration of [^{14}C]dopamine, non-ionic contrast media, iopamidol, was injected into the carotid artery of Mongolian gerbils. The numbers on the left refer to 3 different phases according to reference 9. The numbers on the bottom refer to animal numbers.

Fig. 5. Effect of Iohexol on Localization of Photodensity over Frontal Sections of the Brains 1 min after i.v. Injection of [^{14}C]Dopamine

One min before i.v. administration of [^{14}C]dopamine, non-ionic contrast media, iohexol, was injected into the carotid artery of Mongolian gerbils. The numbers on the left refer to 3 different phases according to reference 9. The numbers on the bottom refer to animal numbers.

was performed for these three areas. The results are summarized in Table II.

Further experiments were undertaken using three Mongolian gerbils administered doses twice as high as the previous experiments. Quantitative autoradiographs of the frontal brain sections from these animals are shown in Figs. 3—5 and demonstrate the extent of damage caused to the BBB. In Fig. 3, which shows the results of ioversol injection, the red and yellow colored areas mark the blood vessels in the bisemispherical brain sections from these

animals numbered No. 1, 2, and 3. Blue or green colored areas represent a very low radioactivity. No marked differences were observed between the left side and the right side of the brain. Contrary to the experimental data of ioversol, differences were found between right and left semispheres in all three animals following iopamidol injection. Animals No. 1 and 2 showed an apparently more severe damage than animal No. 3 (Fig. 4). Animal No. 3 did not show any damage to the BBB, except for the surface of the left lateral cerebral cortex. With iohexol, all

TABLE III. Ratio of Relative Intensity of Radioactivities in the Left Brain to That in the Right Brain

Test drug	Region	Animals			Means	S.D.
		1	2	3		
Ioversol	Cerebral cortex	0.95	0.80	0.94	0.90	0.08
	Hippocampus	0.93	1.21	1.05	1.06	0.14
	Brain stem	1.02	0.99	1.07	1.03	0.04
Iopamidol	Cerebral cortex	1.61	1.35	1.20	1.39 ^{b)}	0.21
	Hippocampus	1.34	1.69	1.66	1.56 ^{a)}	0.19
	Brain stem	1.40	1.39	1.40	1.40 ^{b)}	0.01
Iohexol	Cerebral cortex	2.22	1.25	1.37	1.61 ^{a)}	0.53
	Hippocampus	3.18	4.38	2.21	3.26 ^{a)}	1.09
	Brain stem	2.12	4.25	1.60	2.66 ^{a)}	1.40

a) Significant difference from ioversol treated group ($p < 0.05$). b) Significant difference from ioversol treated group ($p < 0.01$).

three animals showed severe damage. Not only the hippocampus region but also the cerebral cortex and brain stem of the left semispherical brain of the three exhibited red or yellow areas in the pictures with animals Nos. 1 and 2 showing the most severe damage. The ratios of relative intensity, $[(PSL - BG)/S]$ (BG: background, S: square), of the left semispherical brain section to that of the right semispherical brain section are calculated and the results are summarized in Table III. They indicate the extent of damage to the BBB caused by each of the test agents, the value 1.00 indicating no damage. The values for ioversol are approximately 1.00 for all three regions studied. This suggests that ioversol caused practically no damage to the BBB. By contrast, iopamidol administration resulted in ratios of 1.39 or above for the three critical regions. In the case of iohexol, severe damage extended over the entire left semispherical side, with a ratio of 1.61 or above.

DISCUSSION

Intra-arterial infusion of iodinated contrast media, in spite of the potential toxicity, is still the best method presently available for the diagnosis of abnormalities of the vascular system. Research efforts have focused on introduction of safer contrast media. Ioversol is a non-ionic water-soluble compound developed as a low-osmolality iodinated contrast medium. In this report, the neurotoxicity of iodinated contrast media was studied based on the damage to the BBB with leakage of $[^{14}C]$ dopamine into cerebral parenchyma.

Damage of the BBB was observed in the left brain of the animals dosed with meglumine sodium amidotrizoate, iopamidol and iohexol, with a particularly high radioactivity distribution noted in the hippocampus and brain

stem in the iohexol-dosed group. The regions with a high local distribution of radioactivity even 1 min after administration of the test drug are considered to be those with BBB damage due to the administered drug. In the ioversol-dosed group, $[^{14}C]$ dopamine was not taken up into the cerebral parenchymatous cells, an observation similar to that made in the physiological control group. Although the damage grade of the BBB was similar among the three animals receiving ioversol (Table III), a marked difference in this grade was observed with iopamidol among three individual animals. In the iohexol experiment, the most severe damage to the BBB was found in the three critical brain zones. The results corresponded with the tendency observed in ioversol, iohexol, and iopamidol-treated rats in the previous report.³⁾ The nonionic contrast medium ioversol seems safer even for cerebral angiography in terms of BBB damage than the currently available agents.

The trypan blue extravasation technique has been used as a marker of BBB integrity in order to study the neurotoxicity of intracarotid infusion of contrast media.⁶⁻⁸⁾ Salvesen reported that nonionic metrizamide was less damaging than ionic iothalamate meglumine to the BBB in the rabbit.⁶⁾ Velaj *et al.*⁸⁾ concluded that nonionic iopamidol was safer for cerebral perfusion alterations than the other available compounds. In this report, we studied the neurotoxicity of ioversol based on potential damage to the BBB with leakage of $[^{14}C]$ dopamine into cerebral parenchyma. Dopamine is a representative of the family of monoamine neurotransmitters and its brain uptake index (BUI) is reported to be as low as 3.53.³⁾ In general, the blood content ratio in the whole brain without any bleeding is about 3%.⁴⁾ Therefore, the BUI of 3.3 indicates no uptake of the test compound into the brain intracellular space and cells. The $[^{14}C]$ dopamine extravasation technique is very sensitive and is considered a better technique for studying damage to the BBB.

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