Orbital Drainage of Different Sizes of Colloids in Rabbits: A Dynamic Scintigraphic Study

Umit Beden,1 Oktay Yapici,2 Yuksel Sullu,1 Baris Sonmez,1 and Dilek Erkan1

PURPOSE. To investigate the drainage patterns of radiolabeled colloids of different sizes injected into the orbital cavity in an animal model.

METHODS. Twenty-one orbits of 11 rabbits were included in the study. In group 1, human serum macroaggregates with particle sizes of 10 to 100 μm, labeled with 10 mL of 1480 MBq (40 mCi) technetium pertechnetate Tc 99m (99mTc), were used. In group 2, human serum albumin colloidal particles with particle sizes of 50 to 80 nm, labeled with 5 mL of 740 MBq (20 mCi) 99mTc, were used. In group 3, colloidal tin with particle sizes of 300 to 600 nm, labeled with 9 mL of 1665 MBq (45 mCi) 99mTc, were used. The dynamic acquisition of liver for 10 minutes (120 frames for 5 seconds) in a 128 × 128 matrix was acquired immediately after intraorbital injection and at the end of the second hour.

RESULTS. The liver in groups 2 and 3 and the lung in group 1 were visualized in 10 seconds or less in six, five, and four rabbits, respectively. The injected activity persisted in the orbits in varying percentages in all rabbits at the end of acquisition.

CONCLUSIONS. Intraorbital injections have a great potential for systemic absorption and should not be considered as local pharmaceutical administration. (Invest Ophthalmol Vis Sci. 2008;49:2563–2567) DOI:10.1167/iovs.07-1103

The lymphatic system complements the vascular system in the regulation of tissue fluid balance.1–4 They have distinct features compared with blood vessels, serving mainly in the absorption of fluid and macromolecules exiting the blood capillaries.1–4 The dysfunction of the lymphatic system results in localized or generalized lymphedema, especially with advancing age.1

Lymphatic drainage of the periocular region is restricted anterior to the orbital septum. No lymphatic structures have been demonstrated in the orbit except for the dura mater of the optic nerve and the lachrymal gland.5–10 Thus, the lymphatic drainage of the orbit is still up to debate. It is interesting that no lymphedema occurs in the orbit despite the lack of lymphatic vessels. Additionally, we do not have information regarding drainage of the macromolecules, which require lymphatic transport and are unable to enter the blood vessels within the orbit.

Intraorbital drug injection is a common treatment modality for many ophthalmic disorders, but we still lack basic physiological knowledge concerning the drainage of interstitial tissues in the orbit. Exaggerated systemic response after intraorbital drug injection has been reported, raising concerns about the consideration of orbital injection as local treatment.11–14 Additionally, the development of novel long-acting injectable pharmaceuticals for future use in ocular diseases necessitates better understanding of this mechanism.

Lymphoscintigraphy is based on the principle that radiocolloids and radiolabeled macromolecules of suitable sizes and properties, introduced into appropriate tissue planes, are transported by lymphatics and are localized in draining lymph nodes. This imaging technology provides dynamic and static delineation of the functions and components of the lymphatic system under normal and abnormal conditions. Numerous radiopharmaceuticals have been used for lymphoscintigraphy.15 Technetium pertechnetate Tc 99m (99mTc) human colloidal albumin (nanocolloid), colloidal tin, and sulfur colloid—with particle sizes of 50 to 80 nm, 300 to 600 nm, and 1000 nm, respectively—are frequently used worldwide.16

The purpose of this animal study was to demonstrate the pattern of systemic absorption of large molecules injected into the orbital cavity by using different sizes of radiolabeled colloids.

MATERIALS AND METHODS

This study was approved by the ethics committee for animal rights (no. 2006/08; March 30, 2006) of Ondokuz Mayis University and was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Given that drainage is not supposed to be conducted by lymphatic channels, we intended to evaluate the drainage of such large molecules after injection into the orbit. The rationale behind using three differently sized colloids was to assess the effect of molecule size on this supposedly altered absorption and to assess to which extent orbital drainage deviated from that of other tissues in the body. Twenty-one orbits of 11 rabbits in three groups (seven rabbits in each group) received intraorbital injections of labeled colloids under general anesthesia.17,18

Three different kinds of colloidal pharmaceuticals were used. Group 1 received human serum macroaggregates (TechnēScan Lyo-MAA; Mallinckrodt, Hazelwood, MO) with particle sizes of 10 to 100 μm, labeled with 10 mL of 1480 MBq (40 mCi) 99mTc (MAA). Group 2 received human colloidal albumin particles (Nanocol; Amersham Sorin, Saluggia, Italy) with particle sizes of 50 to 80 nm, labeled with 5 mL of 740 MBq (20 mCi) 99mTc, which is a frequently used molecule in lymphoscintigraphy and sentinel lymph node scintigraphy in daily practice (nanocolloid). Group 3 received colloidal tin (American HealthCare; Little Chalfont, Buckinghamshire, UK) with particle sizes of 300 to 600 nm, labeled with 9 mL of 1665 MBq (45 mCi) 99mTc.

Each of these molecules has different methods of preparation, indicated in the corresponding data sheet, in which the volume and the concentration of the 99mTc used are different. To achieve a similar scintillation response, however, the total dose delivered was the same (400–600 mCi) for each group.

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Rabbits were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (2 mg/kg). The depth of the anesthesia was determined by the observation of somatic reflexes.

A 27-gauge dental needle mounted on a 1-mL tuberculin syringe was used for the injections. A tuberculin needle was used to draw the colloidal radiopharmaceuticals into the syringe, and then the needle was replaced before intraorbital injection with a fresh 27-gauge dental needle to prevent dermal contamination during injection. The volume of each injection was 0.1 mL. Colloids were injected into the inferolateral orbit between the globe and the orbital rim, with gentle pressure over the globe to displace it anteriorsuperiorly to prevent any possible ocular penetration. Inferolateral injection was preferred because superolateral and inferomedial orbital spaces are occupied by the lacrimal gland and lacrimal sac, respectively, and the superomedial orbit is shallow to preclude the delivery of radiotracer into peribulbar and retrobulbar spaces. When the needle was within the orbit, a gentle vacuum was applied through the pistol of the syringe to rule out any possible intravascular access. When in proper position (21–23 mm in depth; 10–12 mm from the orbital apex), injections were performed with simultaneous recording of the radioactivity of the orbit, lungs, and liver by a gamma camera (e-Cam; Siemens, Erlangen, Germany). The first pilot injection was performed with a tuberculin needle instead of a dental needle. After this first injection, colloidal material was observed to reflux from the interpalpebral aperture, leading to our conclusion that the injection was performed to the conjunctival fornix transcutaneously instead of through the retrobulbar space. The tuberculin needle was then substituted by a dental needle for the rest of the study.

Each orbit was used for only one injection to prevent traumatic alteration of the physiology of the microvascular environment, which would, consequently, change the rate and the route of the colloidal absorption. Each rabbit received only one intraorbital injection each time. Injection into the other site was performed at least 1 week later, when the activity of the first injection was totally eliminated; this elimination was demonstrated by a control reading before the second injection. The second injection was not necessarily of the same particle or size, and each injection was considered an individual sample. Intra-dermal, subdermal, intramuscular, and intravenous injections at periocular regions were performed as control cases.

A pinhole collimator with a pinhole aperture of 3 mm in diameter was used for dynamic imaging (5 seconds, 120 frames, 128 × 128 matrix). The central field of view of the pinhole collimator was adjusted to include the injection site, the lungs, and the liver of each rabbit. Acquisition occurred immediately after the injection and after the absorption pattern of radiolabeled colloids was assessed. Late images (5 seconds, 24 frames, 128 × 128 matrix) were acquired at the second hour to calculate the percentage of residual activity retained in the orbit (R), which was calculated by the formula \( R = B/A \times 100 \), where \( A \) is the maximum activity in the orbit at early dynamic imaging and \( B \) is the maximum activity in the orbit at late dynamic imaging.

Statistical analysis was limited to calculation of the mean residual activity (R) and standard deviation. Intergroup or intersubject comparisons were not deemed necessary because the objective was to demonstrate the systemic absorption of each molecular size separately but not the comparison of absorption patterns of different molecules.

**RESULTS**

The liver was visualized in 10 seconds or less in six rabbits in group 2 and in five rabbits in group 3 (Fig. 1). Orbital residual radiocolloidal activity was detected at varying percentages in all rabbits (Tables 1, 2). The lungs in group 1 were visualized at 10 seconds or less in four rabbits (Fig. 2). Residual activities in this group were also observed in varying percentages. The lowest residual activity in this group was 12% (Fig. 2, fourth case); blood was observed at the tip of the injector at the end of the injection, assuming the injection to be intravascular. In all other instances, residual activity was greater (Table 1).

In total, the passage of radiocolloids into systemic circulation in 10 seconds or less was observed in 15 of 21 injections (71%). In each case with end-organ activity, conductance of radioactivity was observed between the injection site and the end organ immediately after the injection, resembling vascular flow (Figs. 1, 2).

There was no systemic absorption after intradermal, subdermal, or intramuscular injections of the periocular region at the end of the 2 hours. After intravenous injection, however, rapid end-organ activity was detected, with truncal activity resembling vascular conductance of radiocolloidal pharmaceuticals to the end organ (Fig. 3). In most cases of orbital injection with rapid end-organ activity, the absorption pattern was similar to that achieved after intravenous injection (Fig. 3). Conversely, the retained residual activity in orbital injections occurred in varying degrees, unlike what occurs during intravenous injection, which was actually zero in the latter instance.

As seen in Table 2, the number of cases with systemic absorption was greatest with the smallest colloids (nanocolloids), and the mean residual activity was lowest in this group. The number of cases with systemic absorption was less with colloidal tin and least with MAA (Table 1). Although it was not possible to assess the absolute effect of colloid size on the absorption rate in such a small sample size, it was obvious that residual activity was lower with smaller colloids, implying greater systemic absorption (Table 2). It was also clear that, albeit to a lesser degree, systemic absorption was still possible with larger colloids (colloidal tin and MAA).

**DISCUSSION**

Lymphatic drainage of the orbit is a matter of debate, and previous studies were unsuccessful in demonstrating lymphatics in the orbital tissues. 5–8, 10, 15, 17–21 The results of our study clearly show that large molecules that cannot pass through the walls of blood vessels are quickly absorbed from the orbital interstitium, where there are no known lymphatic channels, in a way to yield high end-organ radioactivity immediately after injection (unlike in other tissues).
Small veins are permeable to small molecules of 1 to 2 nm, but studies of carbon colloids with diameters of 50 nm have shown that the vessels are impermeable to such large molecules in the absence of inflammatory cytokines, which increase vessel permeability. Hence, molecules of 50 to 100 nm are usually used for lymphoscintigraphy. This is the size of the nanocolloid (the smallest molecule used in our study), and it was usually absorbed by lymphatic channels instead of by blood vessels. Larger colloids were also used to assess to which extent orbital drainage differed from that in other parts of the body.

The observation by Sherman and Ter-Pogassian provided the foundation for lymphoscintigraphy. They demonstrated by radioautography that colloidal 198-gold introduced interstitially into rabbit tissues localized in drainage lymph nodes. Lymphoscintigraphy is based on the principle that radiocolloids introduced into appropriate tissue planes are transported by lymphatics and are localized in drainage sentinel lymph nodes. The rationale for our study was that the drainage of a region with no lymphatic vessel would actually bypass the sentinel lymph nodes because the drainage would not be performed by lymphatic vessels. We think that in such tissue, a better way to assess the drainage pattern of molecules from the interstitial space is to look at other tissues that specifically bind the radiocolloid material (i.e., lungs in group 1, liver in groups 2 and 3). We did not observe any regional or distant lymph node activity. However, hepatic and lung activities were visualized in less than 10 seconds on 15 of 21 occasions. This finding is not inconsistent with the rationale for classical lymphoscintigraphy, which relies only on lymphatic drainage of large molecules. Rather, it suggests that the absorption of such large molecules from the orbital interstitium is altered, unlike other tissues in the body. This absorption pattern is clearly faster than the lymphatic route and more permeable than the classical venous route. Therefore, we suppose that drainage of large molecules in the orbital interstitial tissue has an altered, unique pattern unlike that of other tissues in the body.

Animal experiments using radioactive colloids have yielded conflicting results. Such studies of lymphatic drainage from the orbits suggest communication between retrobulbar spaces of both eyes in addition to retained activity in the orbital tissues for up to 1 week. McGetrick et al. additionally detected a changing incidence of lymph node visualization in different occasions of the same orbit. We detected rapid absorption of radiotracers in 15 of 21 injections. The different absorption rates detected in our study is also consistent with the results of the study by McGetrick et al. This finding may support the idea that the orbit is not a homogeneous space in terms of fluid balance. Some compartments may drain more quickly than others, and this may entrap interstitial fluid and prevent it from diffusing into the tissue planes and intravascular space, which is also experienced by probably all ophthalmic surgeons during orbital local anesthetic injections in some cases. McGetrick et al. additionally have detected the spread of India ink along the connective tissue septa through the superior ophthalmic fissure and optic canal in spite of the absence of any lymphatic vessels. Consequently, the free diffusion of India ink was detected posteriorly and into the contralateral orbit and passed through cavernous sinuses. This finding also supports the result of our study that any material gaining access to the cavernous sinus is clearly in the intravascular compartment, which may explain the rapid acquisition time of the end-organ activity detected in our study.

Fogt et al. identified areas of positive endothelial staining adjacent to inflammation, suggestive of possible lymphangiogenesis during the formation of granulation tissue in the orbit, and proposed that lymph vessels do not usually present in the orbital tissue but are newly formed because of the orbital inflammation, which may originate from blood vascular endothelial cells. However, this kind of immature lymphatic vessel probably cannot provide sufficient drainage of the orbital interstitial tissue if there is no inflammation.

There is substantial evidence of high rates of systemic absorption of drugs injected into the periorbicular region. It has been observed in clinical practice that the systemic response to any injected pharmaceutical into the human orbit is greater than required for it to be considered local treatment. Weijtens has detected high serum levels of dexamethasone after peribulbar injection, comparable to levels achieved by a high oral dose, and has proposed that peribulbar injection is not just local treatment. Additionally, Feldmann-Billard et al. has reported hyperglycemia after subconjunctival and peribulbar dexamethasone injection and stated that peribulbar injection should not be considered a local treatment.

### Table 1. Results of Injections and Orbital Residual Activities in Three Groups

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>MAA (10–100 μm)</th>
<th>Orbital Residual Activity (%)</th>
<th>Nanocolloid (50–80 nm)</th>
<th>Orbital Residual Activity (%)</th>
<th>Colloidal Tin (300–600 nm)</th>
<th>Orbital Residual Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lung</td>
<td>58</td>
<td>Liver</td>
<td>45</td>
<td>Nonvisualized</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Nonvisualized</td>
<td>100</td>
<td>Liver</td>
<td>66</td>
<td>Liver</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>Nonvisualized</td>
<td>100</td>
<td>Liver</td>
<td>15</td>
<td>Liver</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>Lung</td>
<td>12</td>
<td>Nonvisualized</td>
<td>100</td>
<td>Nonvisualized</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>Lung</td>
<td>67</td>
<td>Liver</td>
<td>76</td>
<td>Liver</td>
<td>69</td>
</tr>
<tr>
<td>6</td>
<td>Nonvisualized</td>
<td>100</td>
<td>Liver</td>
<td>58</td>
<td>Liver</td>
<td>20</td>
</tr>
</tbody>
</table>

### Table 2. Number of Systemic Absorptions and Mean Orbital Residual Activities

<table>
<thead>
<tr>
<th>Injections n = 21</th>
<th>Group 1 n = 7</th>
<th>Group 2 n = 7</th>
<th>Group 3 n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA (10–100 μm)</td>
<td>Nanocolloid (50–80 nm)</td>
<td>Colloidal Tin (300–600 nm)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>74 ± 32</td>
<td>58 ± 26</td>
<td>66 ± 32</td>
<td></td>
</tr>
</tbody>
</table>

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modality. Hyperglycemic episodes after such injections may be caused by the fast absorption rate of materials administered into the peribulbar area, as shown in our study; these findings support the results of our study. This enhanced rate of absorption was proposed to be attributed to the rich vascular supply of the orbit.\textsuperscript{11} However, the rich vascular supply of a tissue cannot account for absorption of a large molecule, as detected in our study, that usually cannot pass through the blood vessel walls. Instead, this kind of altered absorption of large molecules probably requires distinct absorption characteristics for the vessel wall.

MAA is used for lung scintigraphy\textsuperscript{26} because of its large molecular size (10–100 μm). Given its size, this molecule usually cannot be absorbed by lymphatic channels, and it is trapped in the lung because it is larger than required to pass through pulmonary capillaries after intravenous injection. Intravenous access is usually a prerequisite in lung scintigraphy performed with MAA because lymphatics and blood capillaries are thought to be impermeable to it.\textsuperscript{26} In our study, however, the systemic absorption of MAA was observed in four of seven instances (one of which was thought to be accidentally intravascular, as stated in Results) with varying residual activity percentages at the injection site.

Our study has some limitations. First, because they are the results of an animal study, these findings cannot be conveniently applied to humans. Instead, this should be considered a pilot study to encourage more detailed human research. Second, we could not ensure that such blind injections were extravascular despite the absence of blood withdrawal, with the vacuum applied through the pistol of the syringe before each injection. Third, the 27-gauge needle may not be suitable for blood withdrawal, and small vessels may collapse on gentle vacuuming to prevent blood withdrawal. However, retaining residual activity at the injection site confounds against possible accidental intravenous access because residual activity after intravenous injection was found to be zero, as stated (indicating the total absorption of radioactive material from the injection site), and the vascular trunk conducting colloid to the end organ (Figs. 1–3) was more prominent after intravenous injection. The high incidence (71%) of end-organ activity also confounded against possible intravenous access. This is a high ratio to be accidental; the other possibility is that almost three of every four orbital injections are delivered into the vessels.

It is possible to obtain different-sized particles of the same colloid in the nanometer range by using filters of different pore sizes. Although it would be ideal to take any one of these particles with different size ranges to perform the experiments of nanocolloidal drainage, we did not have the opportunity to access those kinds of filters. Hence, we had to choose particles of three different sizes. However, our aim was not to compare the orbital drainage pattern of different sizes of particles but the absorption of each molecular size separately.

In conclusion, intraorbital injection of pharmaceuticals has great potential for systemic absorption, in a manner that appears to occur more quickly than classic lymphatic absorption; therefore, it should not be considered a method merely of local administration. This altered orbital vascular physiology, in addition to a rich blood supply, may help in our understanding of basic principles concerning orbital drainage and may guide in the research of further orbital pharmaceutical applications.

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