Neuropathological changes related to the transorbital application of ethyl 2-cyanoacrylate adhesive to the basal cerebral arteries of cats

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The long-term toxic effects of ethyl 2-cyanoacrylate adhesive were evaluated histologically in 25 cats. Fresh medical- or commercial-grade adhesive was introduced transorbitally into the subarachnoid space in the vicinity of the right middle cerebral artery. Three sham-operated animals served as controls. The animals were sacrificed at intervals ranging from 2 days to 6 months. For both medical- and commercial-grade adhesive, neuropathological examination disclosed acute and chronic granulomatous inflammation of the meninges and evidence of severe vascular damage, including vessel wall necrosis, inflammation, thrombosis, and occasionally hemorrhage. Most animals showed cerebral infarcts of variable size in the territories of distribution of the basal arteries which were in contact with adhesive. The results of this study show that ethyl 2-cyanoacrylate is capable of producing severe arterial and parenchymal damage. The risk of its deleterious effects should be weighed against its potential benefits. Clinical experience would suggest that ethyl 2-cyanoacrylate can be used in difficult situations as long as care is taken to protect the brain and local blood vessels.

KEY WORDS • cyanoacrylate adhesive • aneurysm • embolism • thrombosis • infarction • plastic adhesive

For the past several decades, cyanoacrylate adhesives have been used for the reinforcement of cerebral aneurysms not amenable to surgical clipping, as well as for the repair of vascular and dural defects, carotid-cavernous fistulas, and arteriovenous malformations. Severe untoward reactions were observed both clinically and experimentally with the earlier Eastman 910 methyl 2-cyanoacrylate adhesive. A newer compound, ethyl 2-cyanoacrylate, has shown promise as a nearly ideal adhesive in the clinical setting. However, several experimental studies have suggested that it too may be toxic under some circumstances.

The present study was undertaken to assess the possible histotoxicity of ethyl 2-cyanoacrylate by using an experimental model which we believe more closely approximates the actual clinical setting in which these compounds are most often used; that is, for the reinforcement of aneurysms at the base of the brain. In this report, we describe the histopathological changes, as observed at intervals up to 6 months, associated with the application of fresh medical- or commercial-grade ethyl 2-cyanoacrylate to the basal cerebral arteries of cats.

Materials and Methods

Twenty-eight adult cats of both sexes were used in this study. The animals were sedated by intramuscular injection of ketamine hydrochloride (10 mg/kg), then anesthetized by intravenous injection of pentobarbital. Additional pentobarbital was given as needed for maintenance of anesthesia. The animal's head was shaved and prepared with Betadine (povidone-iodine) and then fixed in a head-holder specially made to permit variable extension of the neck. Through a supraorbital incision, the right globe was removed. Under the operating microscope at a magnification of ×10 to ×25, the optic nerve was coagulated and incised in the posterior orbit. A high-speed dental drill was used to remove the posterior wall of the orbit from the medial aspect of the optic canal laterally, beyond the superior orbital fissure. Next, the dura was opened sharply in cruciate fashion. The arachnoid was incised over the internal carotid and middle
Experimental evaluation of acrylate adhesives

TABLE 1

Neuropathological findings in 25 cats at different times after application of ethyl 2-cyanoacrylate adhesive

<table>
<thead>
<tr>
<th>Pathological Findings</th>
<th>Length of Survival</th>
<th>Total Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Days</td>
<td>2 Wks</td>
</tr>
<tr>
<td>no. of cats meninges</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>no abnormality</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acute</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>chronic</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>foreign-body giant cells</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>fibrosis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>old hemorrhage (hemosiderin)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>blood vessels</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>not evaluated</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>no abnormality</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>acute changes</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>necrosis/inflammation</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>thrombosis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>chronic changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>organized thrombosis</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>intimal proliferation</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>medial/adventitial fibrosis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>parenchyma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no abnormality</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>gliosis, microglial proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>infarcts</td>
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<td></td>
</tr>
<tr>
<td>acute</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>organizing or old</td>
<td>2</td>
<td>2</td>
</tr>
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<td>unilateral</td>
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<td>bilateral</td>
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<td>small</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>large</td>
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<td>2</td>
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</tbody>
</table>

cerebral arteries. Great care was taken to avoid subarachnoid hemorrhage or direct injury to vessels and brain.

After careful drying of the operative field with suction, fresh tissue adhesive (0.05 to 0.10 ml) was applied via a No. 22 needle and tuberculin syringe. In 11 animals, medical-grade ethyl 2-cyanoacrylate was injected. In 14 animals, commercial-grade ethyl 2-cyanoacrylate (Aron Alpha) was applied. Three control animals had no adhesive injected. A pledget of Gelfoam was placed over the dural defect, and the wound and eyelids were closed with 5-0 interrupted silk sutures.

The animals receiving the medical-grade adhesive were sacrificed by pentobarbital overdose at intervals of 2 weeks (two cats), 6 weeks (three cats), 4 months (two cats), and 6 months (four cats) after injection. The animals receiving commercial-grade adhesive were sacrificed at intervals of 2 days (two cats), 6 weeks (one cat), 1 month (three cats), 5 weeks (one cat), 6 weeks (two cats), 2 months (two cats), and 4 months (three cats) post-injection. The three sham-operated animals were sacrificed at 3 weeks, 4 months, and 6 months, respectively.

After sacrifice, the brains were removed using every effort to avoid injury to the treatment site. The brains, together with the arteries at the base, were immersion-fixed in 10% buffered formalin and embedded whole in celloidin. Alternate serial coronal sections were stained with hematoxylin and eosin (H & E), and Verhoeff-van Gieson (elastic tissue). Leptomeninges, basal arteries, and cerebrum were evaluated for evidence of histopathological reaction and for the presence of adhesive.

Results

The histopathological changes observed in the 25 animals injected with adhesive and in the three sham-operated animals are summarized below and in Table 1.

Leptomeninges

The presence of adhesive within the basal subarachnoid space in the vicinity of the proximal right middle cerebral artery (MCA) was confirmed histologically in all but five of the adhesive-treated animals. The adhesive was identified as an amorphous, semitranslucent, refractile substance which did not take up either the H & E or elastic tissue stains. All but two cats showed a meningeal inflammatory reaction in the immediate vicinity of the adhesive. With the exception of one cat (sacrificed at 2 days), which had only an acute poly-
morphonuclear inflammatory response, all animals showed chronic granulomatous inflammation, characterized by variable fibrosis and infiltration by mononuclear inflammatory cells (predominantly lymphocytes and histiocytes, with occasional plasma cells). A prominent feature of the inflammatory reaction was the presence of foreign body-type giant cells scattered about the margins of the adhesive (Fig. 1 upper left). These were observed in all but four animals. Hemosiderin-laden macrophages indicative of previous hemorrhage were seen in four animals.

There was considerable variability in the intensity of the inflammatory reaction, although generally the most severe changes were observed in the animals sacrificed between 2 weeks and 2 months. The character and intensity of the inflammation did not appear to be directly related to the type of adhesive used. None of the animals showed a significant meningeal inflammatory reaction in areas remote from the adhesive. Two of the three sham-operated animals showed mild lymphocytic infiltration of the basal meninges; however, no acute inflammation, foreign-body giant cells, or fibrosis was present.

**Blood Vessels**

In nine of the 25 animals injected with adhesive, the large vessels at the base of the brain were densely adherent to the base of the skull and could not be removed for histological evaluation. Vascular changes were present in 13 animals. The most severe abnormalities were observed in two cats which had received medical-grade ethyl 2-cyanoacrylate and were sacrificed.
at 2 weeks: both arteries and veins in contact with the adhesive showed an acute “vasculitis,” with medial necrosis, infiltration of polymorphonuclear leukocytes, and formation of in situ thrombi (Fig. 1 upper right). With one exception, none of the animals sacrificed after 1 month showed acute vascular necrosis; however, 11 showed variable adventitial fibrosis and chronic inflammation, medial fibrosis, and intimal proliferation with luminal narrowing. In one animal, one artery also showed focal acute necrosis and thrombosis, in addition to the more chronic changes observed in other vessels. In four cats, some vessels were occluded by organized recanalized thrombi (Fig. 1 lower left and lower right). The internal elastic lamina in affected arteries was generally intact, but often exhibited focal disruptions or reduplication. These vascular changes involved only vessels directly in contact with the adhesive, and predominantly affected large and medium-sized arteries. Smaller arteries and arterioles in contact with adhesive were thickened and narrowed.

No intrinsic abnormalities were observed in vessels remote from the adhesive; however, in one animal, organizing emboli were present in several distal MCA branches. In three animals receiving commercial-grade adhesive and sacrificed at 1 month and 5 weeks, the blood vessels at the base of the brain were entirely normal despite the presence of an intense meningeal inflammatory response to the presence of adhesive. In all three sham-operated animals, the blood vessels were entirely normal.

**Parenchyma**

All but five of the animals that had received adhesive had recent or organizing infarcts of varying sizes, generally occurring in the territories of distribution of the vessels in contact with the adhesive: usually the right MCA, and occasionally the anterior cerebral artery (ACA). Acute infarcts were present in five animals sacrificed from 2 days to 2 weeks following injection of adhesive. They were characterized by widespread acute eosinophilic necrosis of neurons, pallor and vacuolization of the neuropil, and variable infiltration of polymorphonuclear leukocytes. In two animals, these recent infarcts were extensive, involving both cortex and white matter as well as the deep gray nuclei in the entire territory of distribution of the right MCA, and were associated with a slight right-to-left shift of midline structures. One animal had bilateral large recent infarcts in both the MCA and ACA territories. The other two animals had smaller recent infarcts in the right internal capsule in the territory of the lenticulostriate branches of the MCA.

Fifteen animals, sacrificed 1 to 6 months after injection of adhesive, had organizing infarcts characterized histologically by partial cavitation, infiltration of lipid-laden macrophages, and reactive astrogliosis. In three of these animals, the infarcts were large, involving the entire territory of distribution of the MCA (Fig. 2); in one animal, the infarcts were bilateral. The remaining 12 animals had smaller organizing infarcts, the majority of which involved the deep gray nuclei in the territory of the lenticulostriate branches. In five of the animals with infarcts, the etiology of the lesions could be directly attributed to vascular thrombosis, since recent or organizing thrombi were observed in the proximal MCA and/or ACA supplying the infarcted regions. In seven of the cats with infarcts, no thrombi were observed within the arteries of supply at the base, although adventitial inflammation and luminal narrowing were frequently present. In the remaining eight animals with infarcts, the large vessels at the base of the brain were not available for examination. No infarcts were present in the three sham-operated animals.

Parenchymal damage related to direct contact of tissue with adhesive was observed in 13 animals, all of which were sacrificed 1 month or later after injection of adhesive. These changes principally involved the subpial regions of the inferior frontal lobes and consisted of a mild to moderate proliferation of microglial cells, reactive astrogliosis, and focal perivascular lymphocytic cuffing. Similar parenchymal abnormalities were not present in the sham-operated animals.

**Discussion**

There is general agreement that the cyanoacrylate compounds have many properties which make them potentially suitable for use as an adhesive in neurosurgical practice. These include a relatively low viscosity, rapid setting time, good bioadhesiveness, low elasticity, and high tensile strength.

The first of the cyanoacrylate compounds to be regularly used was methyl 2-cyanoacrylate (Eastman 910). Early clinical reports had shown satisfactory results with this compound in the reinforcement of cerebral aneurysms. However, it has now been largely abandoned since several experimental studies have documented histological changes in blood vessels,
meninges, brain parenchyma, and peripheral nerves, attributable to a toxic effect of the adhesive.\textsuperscript{13,17,19,30,32,34} Among the most consistent changes described were: 1) acute and chronic granulomatous inflammation and fibrosis in the meninges; 2) various parenchymal abnormalities, including frank necrosis, inflammation, neuronal destruction, gliosis, and degeneration of axons and myelin; and 3) extensive damage to blood vessels, including adventitial and medial necrosis, thrombosis, aneurysmal dilatation, medial fibrosis, and intimal proliferation. In addition, there were several clinical case reports describing adverse effects believed to be related to the use of methyl 2-cyanoacrylate. Coc and Bondurant\textsuperscript{4} reported the case of a 48-year-old woman who developed angiographically documented occlusion of the proximal left MCA 5 weeks following reinforcement of a left MCA trifurcation aneurysm with Eastman 910 monomer. Sachs, \textit{et al.}\textsuperscript{25} described a 64-year-old woman who died 3 days after reinforcement of a left MCA trifurcation aneurysm with methyl 2-cyanoacrylate. At postmortem examination she was found to have a massive subarachnoid hemorrhage in the Sylvian fissure, with evidence of rebleeding from the dome (which had been coated with adhesive). Histological examination revealed acute necrosis and marked inflammation of the wall of the aneurysm. The authors commented on the similarity of these changes to those described in experimental animals treated with the adhesive. Tsuchiya, \textit{et al.}\textsuperscript{30} reported two patients who developed hemorrhagic infarcts in the distribution of MCA branches following the reinforcement of cerebral aneurysms with methyl 2-cyanoacrylate; however, no pathological documentation was available in either of their cases.

A newer cyanoacrylate, ethyl 2-cyanoacrylate (Aron Alpha), first began to be widely used in Japan and became available throughout the world in the mid-1960's. Early experimental studies indicated that it had good bioadhesive properties and was associated with little or no tissue reaction.\textsuperscript{7,34} Yodh and Wright\textsuperscript{34} evaluated the effects of this adhesive when it was applied topically to the optic nerve and orbital cortex of cats and rabbits. Histological examination was performed at 3, 6, and 12 months after application, and revealed dural and leptomeningeal fibrosis, with minimal inflammation and no damage to blood vessels or parenchyma. These results were corroborated by Chou, \textit{et al.}\textsuperscript{7} who found "minimal" histological changes 3 years following topical application of ethyl 2-cyanoacrylate to the cerebral cortex and femoral neurovascular bundle of cats. None of their animals developed neurological deficits during the 3-year follow-up period. Specific mention was made in their report of the absence of necrosis or other reactive changes in blood vessels.

Despite these initially encouraging results, there have been other experimental studies that have suggested that ethyl 2-cyanoacrylate may cause more tissue damage than previously believed. Lehman and Hayes\textsuperscript{30} evaluated the effects of relatively large amounts (approximately 0.2 ml) of ethyl 2-cyanoacrylate applied to the cerebral cortex and optic chiasm of dogs and primates, respectively. Histological examination performed on animals sacrificed up to 12 weeks after application showed evidence of severe tissue reaction in the leptomeninges, cerebral cortex, optic chiasm, and basal cerebral arteries. The changes in the arteries of the circle of Willis were especially prominent and included intimal proliferation with severe narrowing or total obliteration of the lumen, thrombosis, and mural necrosis with destruction of the tunica media.

More recently, Diaz, \textit{et al.}\textsuperscript{11} studied the effects of 9-year-old medical-grade and of recently acquired commercial-grade ethyl 2-cyanoacrylate topically applied to the cerebral cortex and femoral neuromuscular bundle of cats. Histological examination was performed on animals sacrificed up to 14 days after application. The major pathological findings included acute meningeal inflammation and "necrosis," neuronal and axonal degeneration, vascular wall necrosis, and thrombosis. The histological abnormalities were similar, regardless of whether medical- or commercial-grade adhesive was used. Zumpano, \textit{et al.}\textsuperscript{35} studied the histotoxic effects of medical-grade ethyl 2-cyanoacrylate applied topically to the cerebral cortex of rabbits. Histological examination carried out in animals sacrificed at 4 and 10 days after application revealed extensive necrosis of the superficial cortex, with macrophage infiltration, gliosis, and endothelial proliferation observed at the 10-day interval. Meningeal fibrosis was also present. No thrombosis or vascular wall necrosis was observed in large vessels, but smaller vessels showed endothelial and adventitial proliferation.

In our present study, the major abnormalities observed included acute and chronic granulomatous inflammation of the meninges; severe vascular damage, including vessel wall necrosis, inflammation, thrombosis and hemorrhage; and cerebral infarction. Our findings are in general agreement with the observations previously made by Lehman and Hayes\textsuperscript{30} and Diaz, \textit{et al.}\textsuperscript{11} As in the latter study, we observed no major differences between the medical- and commercial-grade forms of the adhesive with respect to the type and severity of the tissue reaction. The severity of the histological changes affecting the blood vessels was an important finding. The resemblance of these vascular changes to those described in earlier studies with methyl 2-cyanoacrylate is quite remarkable.\textsuperscript{13,17,30,34} It is noteworthy that the vascular damage occurred within a relatively short time following application of the adhesive and produced histopathological changes that were, for the most part, irreversible. We believe that the vascular damage is most likely related to a direct toxic effect of the adhesive on blood vessel walls, although the severe acute inflammatory reaction occurring in the surrounding subarachnoid space may play an important contributing role.

The obvious sequela of these vascular changes was the presence of cerebral infarcts of various ages and

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sizes in the territories of distribution of the basal arteries in contact with the adhesive. The presence of recent or organizing thrombi in these vessels would suggest that the majority of these infarcts were the direct result of vascular occlusion. However, in some cases infarction could also have resulted from vasospasm secondary to either the inflammatory process or a direct toxic effect of the adhesive. In contrast to the fairly extensive tissue damage occurring secondary to vascular compromise, the parenchymal changes resulting from direct contact of the adhesive were much less severe.

Our study indicates that, in its present form, neither commercial- nor medical-grade ethyl 2-cyanoacrylate can be considered an “ideal” bioadhesive with regard to its histotoxicity. When using such adhesives for aneurysm reinforcement, sufficient care must be taken to avoid contaminating major parent vessels feeding the aneurysm. It has been suggested that the induction of a tissue reaction in an aneurysm by the adhesive may be advantageous in that the ensuing fibrosis may actually strengthen the aneurysm wall. However, it should be emphasized that the initial tissue reaction in vessel walls is that of inflammation and necrosis, which in the case of an aneurysm could lead to rupture and rebleeding before a fibrous response has had time to develop. This could also lead to thrombosis of the aneurysm, which could potentially give rise to distal emboli and possibly infarction. Because of the threat of arterial injury and thrombosis, ethyl 2-cyanoacrylate may also be hazardous for the repair of arterial defects or arteriotomies. Injection of the material into the cavernous sinus to obliterate carotid cavernous fistulas likewise carries a risk of vascular or cranial nerve injury.

Nevertheless, animal experiments with tissue adhesives may not be directly comparable to the clinical setting. The overall clinical experience with ethyl 2-cyanoacrylate for aneurysm coating has not included frequent instances of clinically significant histotoxicity. This may be due to the care used during surgery in applying the adhesive only to the aneurysm, with avoidance of application to vessels and sensitive neural structures. There have been several reports of arterial occlusion following the use of ethyl 2-cyanoacrylate for aneurysm reinforcement. However, pathological documentation is lacking in these cases, and thus the evidence implicating the adhesive is largely circumstantial.

Regarding the effectiveness of Aron Alpha reinforcement, the material appeared strongly adherent to basal arteries and brain in 20 of the 25 cats in our study. The material was not found adherent in five cats, and thus ineffective reinforcement may have occurred, as reported by others. Adherence of cyanoacrylates to tissue may be poor, especially if tissues are not carefully dried prior to application. However, adherence may not be necessary for protection against aneurysmal rupture if complete encasement can be achieved. Good clinical results support this concept. Experience at the University of Minnesota with 45 aneurysms invested with ethyl 2-cyanoacrylate led to a single case of postoperative hemorrhage, which was attributed to incomplete encasement. Hayes and Leaver reported that complete encasement of 40 aneurysms with methyl methacrylate (which does not adhere) was followed by no hemorrhagic episodes in 3 to 7 years of follow-up evaluation. Thus, the data suggest that encasement of an aneurysm with tissue adhesive material is usually effective despite non-adherence, providing that encasement is complete.

Conclusions

Ethyl 2-cyanoacrylate tissue adhesives (either fresh medical grade or commercial grade) can evoke a marked histotoxic reaction in meninges, brain parenchyma, and blood vessels. Nevertheless, clinical experience would suggest that these adhesives can still be used in difficult situations as long as sufficient care is taken to protect the brain and local vessels. Such adhesives may be effective in preventing aneurysmal rupture if encasement is complete, even if adherence is not complete. The risks of their use should be weighed against the risk of rupture and the effectiveness and risks of alternative therapies.

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