The Effect of Methylene Blue on Neural Tissue

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Administration of prilocaine hydrochloride is followed by methemoglobin formation. Use of a mixture of prilocaine and methylene blue could prevent methemoglobinemia. The safety of such a mixture for epidural injection was studied in vitro and in vivo. Four isolated frog sciatic nerves were exposed to 0.25 or 0.5 per cent solutions of methylene blue. Conduction velocity was not affected. No microscopic changes were seen in three frog sciatic nerves immersed for 24 hours in 1 per cent methylene blue solution. Thirteen cats were given epidural injections of 1 per cent methylene blue, 2 per cent prilocaine, or physiologic saline solutions. Whereas the eight cats which received prilocaine or saline solution did not develop clinical or microscopic neuropathy, the five cats injected with methylene blue became paraplegic. Microscopic examination of their spinal cords showed inflammation of leptomeninges, nerve roots and myelin sheaths, and swelling of axons. Epidural administration of methylene blue to man appears contraindicated.

(Key words: Methemoglobinemia; Methylene blue; Prilocaine; Epidural anesthesia.)

Methemoglobinemia can be produced by a variety of pharmacologic agents. Prilocaine, a relatively new local anesthetic agent that has the advantage of a low systemic toxicity,1-3 was found to cause methemoglobinemia. This tends to restrict its usefulness for anesthetic techniques, such as continuous epidural analgesia, where large amounts of local anesthetic may be administered for varying time periods.

Although prilocaine-induced methemoglobinemia can be effectively treated with intravenous methylene blue,5-7 its prevention would be preferable. In this context the question arises whether a mixture of prilocaine and methylene blue could be used for epidural administration. If absorption of the anesthetic and the methemoglobin-reducing agent from the epidural space occurred simultaneously the occurrence of methemoglobinemia could be prevented and the clinical usefulness of prilocaine increased. Injection of methylene blue into the epidural space has not been previously reported. Small amounts (1-10 ml) of methylene blue have been injected into the dural sac for diagnostic purposes in cases of posttraumatic rhinorrhea. Following this procedure, rare instances of transient or permanent neurologic sequelae, such as paresis of the upper extremities, cauda equina dysfunction, or ascending paraplegia, have been observed. They were considered to be either allergic or toxic manifestations of methylene blue.5-7 The present study was done to evaluate the safety of epidural injection of methylene blue, especially in view of the relationship between the epidural and subdural spaces.8

Methods

Isolated Nerve Studies

In each of four experiments an isolated frog sciatic nerve, approximately 10 cm in length, was suspended in a nerve chamber on silver-silver chloride stimulating and recording electrodes. Oxygen, led through a nebulizer containing Ringer's solution, filled the chamber with mist, keeping the nerve viable. A 1.5-cm portion of the nerve between the stimulating and recording electrodes was bathed in a cup
containing Ringer’s solution (pH 7.15). The nerve was stimulated with rectangular pulses, 2–8 volts in intensity and 0.1 msec in duration, at a frequency of 25/sec. Elicited compound action potentials were visualized on a cathode ray oscilloscope and photographed. After the potentials had been observed for a 60–100-minute control period, the Ringer’s solution was drained from the cup and replaced by a mixture of methylene blue and Ringer’s solution, buffered to pH 7.0 with sodium hydroxide. Two nerves were exposed to a 0.25 per cent solution of methylene blue, the other two to a 0.5 per cent solution. The action potential was then observed for 30 minutes more to detect the effect of methylene blue upon amplitude, form, and conduction velocity. The experiment was concluded by replacing the methylene blue with Ringer’s solution for subsequent observation.

Four additional sciatic-peroneal nerves were dissected intact from frogs. Immediately after isolation one nerve was immersed in physiologic saline solution, pH 7.0, one in 1 per cent methylene blue solution with a pH of 3.2, and two in buffered 1 per cent methylene blue solution with a pH of 7.2. After 24 hours of immersion the nerves were fixed in 10 per cent formalin solution, stained, and examined microscopically.

**In Vivo Studies in the Cat**

Thirteen cats underwent lumbar laminectomy for the introduction of an epidural catheter, approximately a week before the experiment. They were divided in four groups, each to receive epidural injections of one of
Fig. 2. Normal lumbar spinal cord. This arachnoid membrane is seen at the top of the photograph, with normal dorsal nerve roots present in the subarachnoid space. The dorsal column of the spinal cord is in the lower half of the photograph. Myelin sheaths in the latter and in the nerve roots are unstained and are seen as clear halos about the axons. Hematoxylin and cosin, ×190.

The following solutions in 1.5-ml doses: 1 per cent methylene blue (pH 3.2); 1 per cent methylene blue (pH 7.0); 2 per cent prilocaine (pH 7.0); physiologic saline solution (pH 7.0). One animal received a single injection of 1 per cent methylene blue with an uncorrected pH of 3.2; another cat was injected five times with this solution, i.e., twice daily for two and a half days. Three cats were similarly treated with five injections of methylene blue at a pH of 7.0. Prilocaine 2 per cent and physiologic saline solution were administered to four cats each, twice daily for four days. All cats were closely observed for motor function after injection. They were sacrificed within 24 hours after completion of
the series. At necropsy, the position of the epidural catheter was verified, and the spinal cord removed in toto for fixation in 10 per cent formalin solution. Serial sections of the cord were stained, using a variety of methods, and studied microscopically.

Results

Application of 0.25 or 0.5 per cent methylene blue, pH 7.0, to isolated frog sciatic nerves did not alter form or amplitude of the compound action potential, or change conduction velocity.
Fig. 4. Dorsal nerve root showing severe inflammation. Single arrows at top right and left indicate clusters of polymorphonuclear leukocytes in nerve fibers. Markedly swollen myelin sheaths appear as large clear halos about the axons (double arrow), some of which are degenerating. Hematoxylin and eosin, X405.

Microscopic study of the four sciatic nerves bathed for 24 hours in methylene blue or physiologic saline solution showed normal myelin sheaths, Schwann cells and endoneurial cells; most axons were well preserved. No pathologic changes attributable to methylene blue were seen.

In the cat, epidural injection of physiologic saline solution produced no untoward effects. Motor paresis of the hind legs developed in four cats which had received epidural injections of prilocaine. Motor paresis occurred within one to two minutes after each injection, and lasted 60 to 90 minutes. The pinch reflex was not entirely eliminated. Recovery was complete before each subsequent injection.

All five cats which received methylene blue (pH either 3.2 or 7.0) showed abnormal response. Four of these cats became agitated immediately following the second injection. Throughout the remainder of the experiment they were reluctant to move, exhibiting varying degrees of paraplegia, dragging their hind legs, and not being able to stand. They were sacrificed after the fifth injections. The fifth cat in this group developed flaccid paralysis of the hind limbs, evident five minutes after the first injection. The paralysis persisted, and no additional methylene blue was injected. Necropsy of this cat showed that the tip of the catheter was located in the subdural space at the level of the fifth lumbar vertebra. In each of the other 12 cats the catheter tip was found in the epidural space, at the same vertebral level.

Gross examination of spinal cords of the five cats injected with methylene blue showed blue discoloration of dura, leptomeninges and spinal cord. On serial cross section of the cord the white matter was stained dark blue, and the gray matter, pale blue. Microscopic examination gave a uniform picture. There was acute inflammation of the leptomeninges and nerve roots, characterized by polymorpho-
nuclear infiltration, deposition of fibrin, and localized necrosis of the dura. Swelling and inflammation of myelin sheaths was prevalent, as was axonal swelling in the anterior and posterior roots and grey matter. The pathologic changes usually were more prominent along the posterior and lateral aspects of the spinal cord close to the position of the epidural catheter.

Gross and microscopic examination of the spinal cords of the eight cats which had epidural injections of physiologic saline or prilocaine solution showed no significant changes. Dura, leptomeninges, nerve roots, blood vessels, grey and white matter were normal throughout the length of the spinal cord.

Discussion

Methemoglobinemia which results from administration of prilocaine can be readily treated with small intravenous doses of methylene blue. Despite the ease and effectiveness of this therapy, it is, at least in theory, better to prevent methemoglobin formation. Epidural injection of prilocaine hydrochloride mixed with methylene blue therefore would appear of value. However, results of the present investigation demonstrate that methylene blue deposited in the epidural space in the cat can produce severe neural damage. Both buffered and unbuffered methylene blue produced the same lesions. These findings suggest that the neurologic sequelae following subdural injection of methylene blue reported in man were caused by direct action of this substance, and were inflammatory in character.

It is remarkable that methylene blue caused no pathologic changes in the isolated frog nerve, irrespective of acidity. Even the sensitive measurement of conduction velocity did not show an appreciable effect of exposure to methylene blue. Here, buffered methylene blue solutions had to be used, because axonal conduction in isolated frog nerves is influenced by the pH of the bathing solution.

We do not know why a stain that has been called supravital and generally considered safe should cause such severe inflammation of the spinal cord. Because of this inflammation, epidural administration to man of mixtures of prilocaine and methylene blue is contraindicated.

References