Alteration of Hemoglobin Measurement by Fluorescein

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Sodium fluorescein is an orange-red, water-soluble dye that emits a green fluorescence when exposed to ultraviolet light. The dye can be used to evaluate the adequacy of tissue blood flow and tissue viability. When administered intravenously, fluorescein readily diffuses from the capillaries and is rapidly distributed throughout the extracellular fluid. To determine tissue viability, the area in question is observed for fluorescence under ultraviolet light after intravenous injection of fluorescein.

We observed that hemoglobin concentration, as measured by the OSM2 Hemoximeter, was falsely elevated in a patient who received fluorescein intraoperatively. The following case report demonstrates the apparent increase in hemoglobin concentration after fluorescein. An evaluation of the accuracy of hemoglobin measurements in blood samples containing fluorescein is also presented.

REPORT OF A CASE

A 79-year-old woman was scheduled for a radical neck dissection, hemiglossectomy, and pectoral myocutaneous pedicle flap for treatment of squamous-cell carcinoma of the tongue. After insertion of intravenous and radial-atriary catheters, anesthesia was induced with morphine and diazepam. The trachea was intubated and anesthesia was maintained with enflurane, nitrous oxide, and oxygen. After induction, hemoglobin was 12.3 g/dl as measured by the OSM2 Hemoximeter. During the surgical procedure, a blood loss of about 250 ml was replaced with lactated Ringer’s solution. Immediately prior to the administration of fluorescein, 1 g iv, hemoglobin was 12.0 g/dl. The perfusion of the tissue flap was assessed 15 minutes later and, although no blood had been transfused, the hemoglobin concentration in a sample taken at this time was reported to be 15.0 g/dl. The hematocrit of the same blood specimen was 35 per cent. Additional hematocrit determinations during the remainder of the operation and with the patient in the recovery room showed no change.

METHODS

To assess the effect of sodium fluorescein on hemoglobin concentration as measured by the OSM2 Hemoximeter, various amounts of fluorescein sodium were added to 10-ml aliquots of blood by use of a micropipette. All blood came from one well-mixed unit of human blood in citrate-phosphate-dextrose solution. The maximum volume of fluorescein added to the 10-ml samples was 0.08 ml. All measurements were made on two samples in duplicate, and the four hemoglobin values obtained were averaged. Similar measurements of hemoglobin were made using the 282 Co-oximeter.

RESULTS

The hemoglobin concentration of the blood without fluorescein was 11.1 g/dl. The hemoglobin concentrations measured with the OSM2 Hemoximeter after the addition of fluorescein are shown in figure 1. As the concentration of fluorescein in the blood was increased, the hemoglobin concentration as measured by the OSM2 Hemoximeter increased linearly. When fluorescein (100 mg/ml) without blood was injected into the OSM2 Hemoximeter, a hemoglobin concentration of 47.9 g/dl was reported. When hemoglobin concentrations were measured with the 282 Co-oximeter there was no change after the addition of fluorescein dye to the sample.

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Fig. 1. Hemoglobin concentrations as measured with the OSM2 Hemoximeter with fluorescein added to the sample ($r = 26.0x + 11.4$, $r = .99$).

**DISCUSSION**

Fluorescein is commonly administered intravenously to assess vascular supply and viability of tissues. This dye increases the measured hemoglobin concentration when determined with the OSM2 Hemoximeter but not with the 282 Co-oximeter. The OSM2 Hemoximeter measures the absorbance of the sample at 505 nm after the erythrocytes have been ultrasonically hemolyzed. The absorbance of light at 505 nm is the same for both hemoglobin and oxyhemoglobin, and thus the total hemoglobin concentration can be calculated from the absorbance measured at this wavelength. The maximum absorbance of light by fluorescein is at 493.5 nm, which is very close to the wavelength of light used by the OSM2 Hemoximeter. As the concentration of fluorescein in blood increases, the absorbance of light by the sample increases, and the hemoglobin concentration as measured by the OSM2 Hemoximeter is greater than the actual concentration.

The amount of error in the hemoglobin measurement after fluorescein with the OSM2 Hemoximeter is dependent on the serum fluorescein concentration. The intravenous dosage of fluorescein is 10 mg/kg in Caucasian patients, but two to three times this amount may be needed to observe fluorescence in patients with darker skin. After intravenous administration, fluorescein is rapidly distributed throughout the extracellular fluid and is excreted unchanged in the urine and bile. In studies in rabbits, the concentration of fluorescein in blood decreased exponentially, with a half-life of about 28 min. Since the concentration of fluorescein in blood varies according to the dose injected, the extracellular fluid volume, and the degree of clearance by the kidneys and liver, the size of the error in hemoglobin measurement depends on many factors. With normal clearance, serum fluorescein levels are sufficiently low after three hours that hemoglobin measurement should not be affected.

There was no increase in the hemoglobin concentration with fluorescein when measured with the 282 Co-oximeter. This instrument measures the absorbance of the sample at multiple wavelengths, the lowest of which is 535 nm. This wavelength is sufficiently above that of fluorescein's maximum absorbance that there is minimal light absorbance by the dye and no interference with the hemoglobin measurement.

Other photometric devices for measuring hemoglobin were not tested, but analyzers that measure the absorbance at wavelengths near that of fluorescein's maximal absorbance should report inaccurate hemoglobin concentrations when this dye is used. If hemoglobin measurements are made in samples of blood from patients who have received fluorescein, one needs to know whether the dye will interfere with the measurements in the analyzers that is used. Centrifuged hematocrits will not be affected by the dye and are useful when hemoglobin measurements are not accurate.

These data demonstrate that fluorescein may produce inaccurate hemoglobin measurements with one photometric analyzer. In samples of blood from patients receiving fluorescein, hemoglobin concentrations should be measured with analyzers not affected by the dye or, alternatively, hematocrits should be measured. Any unexplained increase in hemoglobin concentration after fluorescein injection may be due to interference by the dye with the hemoglobin measurement.

**REFERENCES**