The effect of phototherapy on amino acid solutions containing multivitamins


Recent evidence indicates that riboflavin, a photosensitizer, can enhance the reduction of serum bilirubin in neonates receiving phototherapy. Concern has been expressed, however, since in vitro studies have demonstrated alteration in DNA which may (or may not) become mutagenic. We are concerned that many low-birth-weight infants receiving phototherapy also receive parenteral protein solutions containing photosensitizers in the form of multivitamin additives. Our concern stems not only from the standpoint of riboflavin-DNA alteration but also centers on the effect of the photosensitizer-enriched infusate on the amino acid composition when exposed to direct irradiation in the 420 to 470 nm waveband of the visible spectrum. The effect of such irradiation on photosensitizer-containing amino acid solutions forms the basis of this report.

MATERIALS AND METHODS

A solution of crystalline amino acids (Freamine II, McGaw Labs, Glendale, Calif.) was mixed with 10% dextrose to provide the protein equivalent of 0.5 gm/dl. One milliliter of multivitamin solution (M.V.I., USV Pharmaceutical Corp, Tuckahoe, N.Y.) was added to 500 ml of this solution. This calculated to provide nearly a magnitude greater concentration of riboflavin than that observed to enhance bilirubin photodecomposition in vitro. The solution was then passed through a 0.25 millipore filter under a laminar flow hood to ensure no bacterial contamination. The experiment was begun as soon as the solution was prepared and was continued for exactly 24 consecutive hours. Cultures taken from the collection reservoir upon completion of the experiments were found to be sterile.

Amino acid solutions with and without M.V.I. were exposed to constant photoirradiation during a continuous flow setup to a theoretical infant weighing 1.5 kg calculated to receive 2.0 gm protein/kg/24 hours at a rate of 0.156 ml/minute using a constant infusion pump. Amino acid solutions similar in all aspects to the above, but with no flow, were also exposed to constant photoirradiation.

All parts of the reservoir bottle, infusion tubing (pre- and post-cubator) and collection bottle, were shielded from light by heavy aluminum foil. This assured that only a 20 cm length of intravenous tubing inside the incubator was exposed to light. Control solutions were prepared at the same time as test solutions and contained M.V.I. They were protected from light at all times.

Light energy was delivered via a canopy (Air Shields, Hatboro, Pa.) containing a bank of 8 GE F20T12/B bulbs placed immediately over the incubator (Air Shields Isolette). A Plexiglas shield was in position between the light source and the walls of the incubator. This should have provided a more than adequate barrier to near UV fluence which could react with flavins. Flux was monitored at 800 to 1,000 W/cm²/nm from the 420 to 470 wavelength of the visible spectrum using an IL 155 radiometer (International Light, Newburyport, Mass.).

Amino acids were analyzed on an automatic JEOL analyzer using a one-column system. Because of resolution problems, tryptophan was analyzed separately on a Beckman amino acid analyzer.

RESULTS

Amino acid analysis from the lot number studied was performed to compare our concentrations with those expected from the manufacturer's label. We were in agreement with all amino acid concentrations analyzed with the exception of cystine, which was negligible in all samples (< 0.02 gm/ml).

There was no change in amino acids from solutions containing M.V.I. when exposed to photoirradiation as...
Table. Effect of 420-470 nm light and M.V.I. on amino acid solution*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Light + M.V.I. Mean (± SD)</th>
<th>% Decrease</th>
<th>Light only Mean (± SD)</th>
<th>% Decrease</th>
<th>Controls Mean (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>2,613 (± 566)</td>
<td>40.2</td>
<td>3,860 (± 431)</td>
<td>11.7</td>
<td>4,371 (± 526)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>498 (± 350)</td>
<td>44.0</td>
<td>517 (± 19)</td>
<td>49.9</td>
<td>891 (± 24)</td>
</tr>
<tr>
<td>Histidine</td>
<td>1,673 (± 56)</td>
<td>21.9</td>
<td>2,036 (± 62)</td>
<td>5.13</td>
<td>2,146 (± 113)</td>
</tr>
</tbody>
</table>

*M.V.I. = Multivitamin solution (see text).
†All values expressed as μmole/l and represent three separate experiments.

DISCUSSION

Riboflavin is a photosensitizer known to decrease serum bilirubin values in animals and newborn infants when used as a supplement to photoradiation. This process is thought to proceed through the generation of high-energy oxygen radicals (ʻO2) which, in turn, oxidize the bilirubin molecule and render it water soluble. Recent concern over the possible mutational effects on DNA, however, has discouraged the use of riboflavin as an adjunct to phototherapy. Reports of effects of sensitized photo-oxidation on proteins suggests that the principal sites of damage are histidine, methionine, tryptophan, and tyrosine. The photosensitizer and conditions present at the time of photoradiation determine the mechanism and nature of products found. During photo-oxidation the primary amine group of the amino acid may be affected or the imidazole ring may be broken. Photo-oxidation of tryptophan in the presence of protoporphyrin in formic acid resulted in nine products. Likewise, photo-oxidation of methionine with eosin and methylene blue yielded methionine sulfoxide with the initial intermediate product of dehydrodromethionine. In acid conditions the dehydrodromethionine was converted to methionine sulfoxide.

Recent evidence has shown that strong fluorescent light, delivered to human diploid fibroblast cultures in the presence of photosensitizers in vitamins, had an adverse effect. Tryptophan and cystine become highly inhibitory to cell growth, whereas photo-oxidized methionine was highly toxic to the cells. This latter effect was observed only in the presence of riboflavin, folic acid, or biotin. Additional evidence also indicates that normal plasma folate may be reduced by 30 to 50% when exposed to ultraviolet light at 360 nm waveband in vitro.

Further investigation into visible light-induced toxic photoproducts indicates that peroxide (H2O2) generated by riboflavin and tryptophan or by riboflavin and tyrosine accounted for 40% of the toxicity for human cells in culture. Riboflavin and tryptophan are also primarily responsible for nonperoxide toxic products generated.

The data presented herein do not attempt to describe the products of photo-oxidation of those amino acids affected. On the other hand, the appearance of methionine sulfoxide was detected and raises the question whether this product may indeed be a cellular toxic agent.

Exposure of liquid nutrients (amino acid solutions) to light, especially those emitting flux at 420 to 470 nm waveband, is not monitored adequately to ensure that infants are not being deprived of important nutrients as well as perhaps receiving a toxic substance. Although we could demonstrate no change in amino acid + M.V.I. during the continuous flow technique, this may have been due to the strict experimental controls imposed to protect the source bottle from light. Such controls are not the usual circumstance in the nursery. To the contrary, most protein solutions are continuously exposed to unknown quantities of light, which is then intensified if the infant receives phototherapy for hyperbilirubinemia.

We are concerned, therefore, as to the safety of adding photosensitizing agents to amino acid solutions which are delivered to infants under less than controlled light environment. Data presented herein, and reported by others, would lead us to recommend that multivitamin solutions not be added to amino acid solutions but delivered separately and shielded from light.

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REFERENCES


Aicardi syndrome in a male infant

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Aicardi syndrome is a condition characterized by the association of infantile spasms, congenital abnormalities, and female sex. Aicardi knows now of about 100 cases (personal communication, 1978).

The most frequently reported findings in the syndrome are (1) involvement of the central nervous system manifested by convulsions, often as flexion spasms, agenesis of the corpus callosum, characteristic electroencephalographic changes, and mental subnormality; (2) ocular manifestations, which include chorioretinal footprint-shaped lacunae, resulting from zones with depigmentation of the pigment epithelium; additional ocular abnormalities are staphyloma, coloboma of the optic nerve, and microphthalmia; (3) rib and vertebral dysplasias, scoliosis, and other osseous abnormalities.

The most widely accepted view is that the syndrome has an X-linked dominant inheritance, which is lethal in the male.

We report the first observation of a 46,XY male patient with the salient features of the syndrome.

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CASE REPORT

The patient, the only child of healthy nonconsanguineous parents, was referred to the Department of Child Neurology, University of Rome, at the age of 11 months, because of infantile spasms. At conception the mother was 22 years of age. Pregnancy was unremarkable; labor and delivery were spontaneous at term. Birth weight was 3.200 gm. Apgar score was 9 at one minute.

See related article, p. 235.