Nutrition and Cancer

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/hnuc20

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Myron D. Gross a, Thomas D. Bishop b, John D. Belcher b & David R. Jacobs Jr. b

a Div. of Epidemiology, School of Public Health, University of Minnesota, 1300 South 2nd St., Suite 300, Minneapolis, MN, 55454-1015 Phone: (612) 624-5417 Fax: (612) 624-5417 E-mail:
b Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN, 55454-1015


To link to this article: http://dx.doi.org/10.1080/01635589709514521

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Solubilization of β-Carotene in Culture Media

Myron D. Gross, Thomas D. Bishop, John D. Belcher, and David R. Jacobs, Jr.

Abstract: Several methods were evaluated for the solubilization of supraphysiological concentrations of β-carotene in culture media. The addition of β-carotene in ethanol as 0.1% (vol/vol) of culture medium that contained 10% fetal bovine serum and incubation at 37°C for 30 minutes solubilized approximately 50% of the added carotenoid. Solubilized β-carotene concentrations from 3.06 to 36.4 μmol/l were obtained by this method. Ultracentrifugation studies localized the solubilized β-carotene to low-density lipoproteins (67.0%) and high-density lipoproteins (24.9%). Other methods utilizing various amounts of fetal bovine serum, ethanol, and hexane as solvents did not significantly improve the solubilization of β-carotene compared with the use of ethanol as 0.1% (vol/vol) of culture medium that contained 10% fetal bovine serum. Nonetheless, solubilization of supraphysiological concentrations of β-carotene indicated the feasibility of preparing culture media containing physiological concentrations (0.5 μmol/l) of β-carotene.

Methods

Materials

RPMI1600 medium, penicillin-streptomycin, fungizone, and fetal bovine serum were purchased from GIBCO. [14C]β-carotene (sp act 192 μCi/mg) was a generous gift from Hoffmann La Roche. Ecoscint, a liquid scintillation cocktail, was purchased from National Diagnostic. All organic solvents were high-performance liquid chromatography (HPLC) grade.

Carotenoid Purity and Quantitation

The concentration and purity of [14C]β-carotene were verified by HPLC analysis, as described by Bieri and co-workers (6), with a Beckman HPLC System Gold. It consisted of a model 507 autosampler, a model 168 diode array detector module, and a model 116 programmable solvent module. The HPLC column was a Beckman Ultrasphere ODS 4.6 mm × 25 cm 5-μm reverse-phase C18 column with an amine guard column. [14C]β-carotene was identified on the basis of retention time and ultraviolet-visible spectral scans. The carotenoid preparation was >97% pure. Specific activity of [14C]β-carotene was confirmed by liquid scintillation counting with a Beckman LS 3801. Radioactive β-carotene in toluene was diluted in hexane and ethanol. All sample preparation was done under darkroom conditions. Radioactive β-carotene was quantitated on the basis of its specific activity.

Solubility of [14C]β-Carotene in Media

The concentration and purity of [14C]β-carotene were verified by HPLC analysis, as described by Bieri and co-workers (6), with a Beckman HPLC System Gold. It consisted of a model 507 autosampler, a model 168 diode array detector module, and a model 116 programmable solvent module. The HPLC column was a Beckman Ultrasphere ODS 4.6 mm × 25 cm 5-μm reverse-phase C18 column with an amine guard column. [14C]β-carotene was identified on the basis of retention time and ultraviolet-visible spectral scans. The carotenoid preparation was >97% pure. Specific activity of [14C]β-carotene was confirmed by liquid scintillation counting with a Beckman LS 3801. Radioactive β-carotene in toluene was diluted in hexane and ethanol. All sample preparation was done under darkroom conditions. Radioactive β-carotene was quantitated on the basis of its specific activity.

The authors are affiliated with the Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN 55454-1015.
experiments. The mixtures were mixed and incubated at 37°C for 30 minutes. Subsequently, samples were removed and analyzed for their [14C]β-carotene content. The concentration of β-carotene in the media preparation and the percentage of total β-carotene solubilized in the media preparation were determined in the experiments.

Analysis of [14C]β-Carotene Distribution in Culture Media

Media were analyzed by zonal density gradient ultracentrifugation, as described by Belcher and associates (7). Fractions were collected and counted by liquid scintillation.

Results

Several methods were analyzed for the solubilization of [14C]β-carotene in media. Various concentrations of [14C]β-carotene in ethanol or hexane were added to media preparations containing 10% and 20% fetal calf serum. Solubilized [14C]β-carotene concentrations were determined after a 30-minute incubation period at 37°C. The results are shown in Table 1. The method used to introduce β-carotene into media had a significant effect on β-carotene concentrations. An effective method was delivery of [14C]β-carotene in ethanol with a final ethanol-to-media ratio of 1:1,000 (0.1%). Fifty-three to 68% of added [14C]β-carotene was recovered in media, in which the concentrations for 100% solubilization ranged from 5.57 to 68.1 μM. That is, physiological (=0.3–2.0 μmol/l) and supraphysiological (>2.0 μmol/l for most human populations) concentrations were recovered in the culture media.

Other methods were less effective. [14C]β-carotene delivered in hexane (0.1% media, vol/vol) had a recovery in media of 12–39%, for which the concentrations at 100% solubilization ranged from 5.9 to 97.9 μM. An increase in organic solvent to 1% (vol/vol, solvent to media) did not significantly improve recovery. That is, 12–15% and 57.0–76.4% of β-carotene were recovered with a 1% hexane and 1% ethanol delivery, respectively. The addition of media to [14C]β-carotene dried under nitrogen from hexane or ethanol resulted in low recoveries (13–15%, data not shown). The use of a higher proportion of fetal bovine serum (20% vs. 10%) did not affect recovery of carotenoids in hexane or ethanol at 1% or 0.1%. Overall, recovery was more dependent on the method of delivery (ethanol vs. hexane vs. dried down) than the amount of β-carotene delivered to the media. Recoveries were similar for relatively low and high concentrations of [14C]β-carotene for each organic solvent-fetal bovine serum-RPMI combination (Table 1).

In human subjects, plasma β-carotene has been associated primarily with lipoprotein particles; the highest concentrations have been found in low-density lipoproteins (LDL) (8). We analyzed the distribution of [14C]β-carotene in tissue culture media. Analysis was by zonal rate density gradient centrifugation. The concentration of solubilized [14C]β-carotene in media for these experiments was 2.9 × 10−14 mol/l. The distribution of β-carotene across the density gradient was determined by radioisotope counting of collected fractions. Results of the analysis are shown in Figure 1. The vast majority of [14C]β-carotene (67%) was associated with LDL. LDL1 alone was associated with 46.5% of total [14C]β-carotene recovered in the analysis. The next largest fraction of [14C]β-carotene (24.9%) was associated with high-density lipoprotein 3. Relatively small amounts of [14C]β-carotene were associated with very-low-density lipoprotein, LDL2, LDL3, and high-density lipoprotein 2. Overall recovery of radioactivity for the analysis is 91.9%.

Overall, the results confirm the solubilization of β-carotene in culture media at physiological and supraphysiologically

Table 1. Solubilization of [14C]β-Carotene in Tissue Culture Media

<table>
<thead>
<tr>
<th>Media Composition, % (vol/vol)</th>
<th>β-Carotene Solubilized in Media, % of added β-carotene</th>
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<tbody>
<tr>
<td></td>
<td>Ethanol</td>
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<td>10.0</td>
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<td>20.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a: FBS, fetal bovine serum; RPMI, complete culture medium, including 1% penicillin-streptomycin and 1% fungizone but no FBS.
b: Values are means ± SD of no. of observations in parentheses. Values in brackets represent calculated β-carotene concentration with 100% solubilization (100% = complete solubilization). Because several experiments were done with similar, but not identical, amounts of added β-carotene, a range is given for calculated concentration. Percentage of β-carotene is an average of all experiments.

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Several methods for the solubilization of β-carotene in culture media are evaluated in this report. The most effective method is the delivery of β-carotene in ethanol. Approximately 50% of the β-carotene introduced into culture media can be recovered in a soluble form. A similar fraction of the total β-carotene is recovered regardless of the absolute amount (~5 and 65 μmol/l) added to the culture. These results suggest that the partitioning of carotenoids into culture media is a highly time-dependent process. In addition, the solvent used for introduction of β-carotene into the culture media has a significant effect on solubilization. Interestingly, the more hydrophobic solvent, hexane, is less effective than ethanol for the delivery of the carotenoid. This finding may be the result of a slower rate of partitioning between hexane and culture media than between ethanol and the media.

Using ethanol for the delivery of β-carotene provides for the solubilization of physiological (~0.3–2.0 μmol/l) and supraphysiological (>2.0 μmol/l for most populations) concentrations of the carotenoid in culture media. These results indicate that serum-containing media have the capacity for solubilization of a wide range of concentrations of β-carotene. This finding is consistent with clinical observations of human subjects. Supraphysiological concentrations of β-carotene, similar to those solubilized in this report, are found in the blood of patients receiving treatment for erythropoietic protoporphyria.

The majority of β-carotene can be recovered from LDL in the culture media. Smaller amounts are found in other lipoprotein particles. This distribution of carotenoids in culture media is analogous to the in vivo distribution of β-carotene (8). Thus we have successfully solubilized β-carotene in culture media for HL-60 cells and found that β-carotene in the culture is carried on lipoproteins in a manner identical to that found in vivo.

Acknowledgments and Notes

The site of this experimental work is the Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN. This research project is supported in part by a grant from the Biomedical Research Support Program. Address reprint requests to Dr. Myron Gross, Div. of Epidemiology, School of Public Health, University of Minnesota, 1300 South 2nd St., Suite 300, Minneapolis, MN 55454-1015. Phone: (612) 624-5417. FAX: (612) 625-8950. E-mail: Gross@Epivax.Epi.umn.edu.

Submitted 7 February 1996; accepted in final form 16 October 1996.

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