Radioactive Labelling of Lipids in Rat Neurosarcoma by Intravenous Injection of [1-^{14}C]-Octadecenol

Nikolaus Weber, Manfred Volm, and Klaus Wayss

Deutsches Krebsforschungszentrum, Institut für Experimentelle Pathologie, Postfach 10 19 49, D-6900 Heidelberg 1

and

Ingolf Richter, Helmut K. Mangold and Kumar D. Mukherjee

Bundesanstalt für Fettforschung, D-4400 Münster

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Radioactivity from *cis*-9-[1-^{14}C]octadecenol, injected intravenously into rats bearing neurosarcoma, is incorporated to a significantly greater extent into tumor than into muscle. In the lipids of both tissues, radioactivity is incorporated predominantly into the acyl moieties, rather than into the alkyl or alk-1-enyl moieties, of diradylglycerophosphocholines, diradylglycerophosphoethanolamines, and triradylglycerols.

A relationship seems to exist between malignant growth and lipid metabolism [1, 2]. Neoplastic tissues have been shown to contain higher levels of alkoxylipids than normal tissues [3, 4]. Since long-chain alcohols are known to be precursors of the alkyl and alk-1-enyl moieties in the alkoxylipids of mammalian tissues [5, 6], we have studied the incorporation of radioactivity from *cis*-9-[1-^{14}C]octadecenol into neurosarcoma transplanted subcutaneously in rats. Moreover, we have determined the extent and pattern of incorporation of radioactivity into the lipids of neurosarcoma and muscle, in order to explore the possibility of scintigraphic diagnosis of tumors by the use of an alcohol labelled with a γ-emitter.

Neurosarcoma [7, 8] were transplanted subcutaneously in the backs of male Sprague-Dawley rats having an average weight of 200 g. The substrate, *cis*-9-[1-^{14}C]octadecenol, specific activity 1.04 GBq/mmol, was prepared from [1-^{14}C]oleic acid (Amersham Buchler, D-3300 Braunschweig) by reduction of the corresponding methyl ester with $LiAlH_4$ and purified by thin-layer chromatography to a radiopurity better than 99%. Solutions of *cis*-9-[1-^{14}C]octadecenol, 1.85 MBq, in 0.1 ml ethanol, were injected into tail veins of rats. The animals were killed by cervical dislocation at intervals of 1 h, 24 h, 48 h, and 96 h after application of the substrate. Tumors as well as femoral muscle tissues were each homogenized in a mixture of methanol-chloroform (2 : 1), and aliquots were taken for the determination of radioactivity in a liquid scintillation counter (Packard Instrument Company, Downers Grove, Illinois, USA). Subsequently, the lipids were isolated [9] and fractionated by thin-layer chromatography [6, 10]. Alkylglycerols, alk-1-enylglycerols, and long chain alcohols which were obtained by reduction of the glycerophospholipids and neutral lipids using $LiAlH_4$, were also separated by thin-layer chromatography [11]. The distribution of radioactivity among the various lipid fractions was determined by means of a TLC Scanner (BF-Vertriebsgesellschaft, D-7547 Wildbad).

The radioactivity from intravenously administered *cis*-9-[1-^{14}C]octadecenol is extensively incorporated into neurosarcoma as well as muscle tissue of rats, as shown by the results given in Table I. After decline of the initial flux of radioactivity (24 h), the level of radioactivity per mg tissue is found to be slightly, yet significantly higher in neurosarcoma than in muscle throughout the experiment.

The data presented in Table I also show that in both neurosarcoma and muscle the label from the radioactive octadecenol is incorporated predominantly into the phospholipids, however, the relative proportions of radioactivity in the phospholipids are lower in neurosarcoma than in muscle. The label in the phospholipids of both tissues is found almost exclusively in diradylglycerophosphocholines and diradylglycerophosphoethanolamines. Ehrlich ascites cells grown in the peritoneal cavity of mice have also been shown to incorporate the radioactivity from long-chain alcohols mainly into diradylglycerophosphocholines and diradylglycerophosphoethanolamines [5].

It is evident from Table I that the relative proportions of labelled diradylglycerophosphoethanolamines are lower in neurosarcoma than in muscle. In both tissues, the relative proportion of radioactively labelled diradylglycerophosphoethanolamines tends to increase with time while that of labelled diradylglycerophosphocholines decreases. These findings seem to support proposed pathways in which, via base exchange or back reaction of CDP-choline (-ethanolamine), a part of the glycerophosphocholines

Reprint requests should be sent to Dr. N. Weber.

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Table I. Distribution of radioactivity in rat neurosarcoma and muscle, as well as in their phospholipids and neutral lipids after intravenous injection of *cis*-9-[1-^{14}C]octadecenol.

Tissue	Time after injection [h]	Radioactivity in tissue [a] [cpm/mg]	% Radioactivity in:		
			Diradylglycero-phosphocholines	Diradylglycero-phosphoethanol-amines	Neutral lipids [b]
Neuro-sarcoma	1	40 ± 16.5	40	17	37
	24	50 ± 10.6 [c]	48	29	23
	48	37 ± 4.3 [c]	42	33	25
	96	25 ± 4.0 [c]	37	27	36
Muscle	1	56 ± 13.4	29	23	20
	24	37 ± 5.4	45	46	7
	48	30 ± 4.4	38	48	14
	96	20 ± 4.8	35	52	13

[a] Mean of 5 – 9 tissue samples ± SEM; [b] excluding octadecenol; [c] radioactivity significantly higher than in muscle (Distribution-free sign test according to Fisher, $\alpha = 0.01$; confidence interval > 96%).

is converted to glycerophosphoethanolamines and not vice versa [12, 13].

The distribution of radioactivity in the radyl moieties of phospholipids and in the fractions of neutral lipids of neurosarcoma and muscle is given in Table II. In both diradylglycerophosphocholines and -ethanolamines of the two tissues the major proportion of radioactivity is located in the acyl moieties. In the radyl moieties of both phospholipids the proportion of radioactivity in the sum of alkyl and alk-1-enyl moieties is distinctly lower in neurosarcoma than in muscle. Our findings in neurosarcoma are quite different from those reported for Ehrlich ascites tumors, which have been shown to incorporate radioactivity from long-chain alcohols, injected directly into peritoneal cavity, predominantly into the alkyl and alk-1-enyl moieties of diradylglycerophospholipids [5].

The results given in Table II show that in both neurosarcoma and muscle the radioactively labelled diradylglycerophosphocholines contain alkyl but practically no alk-1-enyl moieties. The radioactive diradylglycerophosphoethanolamines in neurosarcoma contain much higher proportions of alk-1-enyl than alkyl moieties, whereas those in muscle contain large proportions of alk-1-enyl moieties at 24 h after injection of octadecenol. In contrast to neurosarcoma, muscle contains fairly large proportions of radioactive alkyl moieties in diradylglycerophosphoethanolamines. The virtual absence of radioactive alk-1-enyl moieties in diradylglycerophosphocholines and a fairly large concentration of radioactive

Table II. Distribution of radioactivity in radyl moieties of phospholipids and in fractions of neutral lipids of rat neurosarcoma and muscle after intravenous injection of *cis*-9-[1-^{14}C]octadecenol.

Tissue	Time after injection [h]	% Radioactivity in radyl moieties of phospholipids						% Radioactivity in neutral lipid fractions				
		Diradylglycerophospho-cholines			Diradylglycerophospho-ethanolamines							
		Alkyl	Alk-1-enyl	Acyl	Alkyl	Alk-1-enyl	Acyl	Radyl-glycer-ols [a]	Chole-sterol	Octa-decenol	Trira-dyl-glycer-ols [b]	Chole-steryl-esters [c]
Neuro-sarcoma	1	13	tr	87	tr	14	86	8	14	14	47	17
	24	8	tr	92	tr	8	92	11	26	1	33	29
Muscle	1	28	tr	72	39	5	56	4	17	58	15	6
	24	14	tr	86	14	35	51	20	29	25	12	14

[a] Including unidentified constituents; [b] including small proportions of unesterified fatty acids; [c] including wax esters; tr = traces.

alk-1-enyl moieties in diradylglycerophosphoethanolamines of both tissues strongly suggest, in accordance with previous findings in intestinal mucosa [6] and Ehrlich ascites tumor cells [5], that alkylacylglycerophosphoethanolamines are selectively desaturated to alk-1-enylacylglycerophosphoethanolamines.

The distribution of radioactivity in the neutral lipid fractions of neurosarcoma and muscle, also given in Table II, shows the presence of octadecenol in both tissues. Considerably lower proportions of labelled octadecenol are found in neurosarcoma as compared to muscle. Radioactively labelled alkyl and alk-1-enyl moieties are not detected in the triradylglycerol fractions in contrast to earlier findings in Ehrlich ascites tumor cells, which have been shown to incorporate large proportions of radioactivity from added long-chain alcohols into alkyldiacylglycerols of the cells [5]. Our results show also that the relative proportion of radioactivity contained in the acyl moieties of triradylglycerols is much higher in neurosarcoma than in muscle. Obviously, radioactive fatty acids formed by oxidation of octadecenol are incorporated into the triradylglycerols of neurosarcoma to a much greater extent than into the triradylglycerols of muscle. The relatively large proportions of radioactivity found in cholesterol and cholesteryl esters of neurosarcoma and muscle must be attributed to degradation of the substrate and synthesis via acetyl-CoA.

The results of our study show that the radioactivity from intravenously administered octadecenol is incorporated predominantly into the acyl moieties of the lipids in both neurosarcoma and muscle, and not into the alkyl and alk-1-enyl moieties, as reported for Ehrlich ascites tumor cells [5]. Although the radioactivity in neurosarcoma is distinctly higher than in muscle, the differences observed in the level of radioactivity in the two tissues may not be an adequate basis for scintigraphic detection of such tumors. It is likely, however, that the use of a more direct precursor of the alkoxylipids, such as an alkylglycerol, which should be labelled with a γ-emitter, *e. g.* ^{11}C, will provide a means for differentiating between neurosarcoma and muscle tissue by scintigraphy.

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