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Original article

Relationship between lipid parameters and the occurrence and severity of lesions in the heart : a study on rats fed low erucic acid rapeseed oil

G. Rocquelin *, P. Juaneda, P.O. Astorg and A. Grynberg
with the technical assistance of M. Degois and L. Guenot

Station de recherches sur la qualité des aliments de l'homme, INRA, 17 rue Sully, 21034 Dijon-Cédex, France

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Summary — Fifty-six male Wistar SPF rats were fed a diet containing low erucic acid rapeseed (LEAR) oil (15% by weight) as the only source of lipids for 18 wk. Lipid parameters (fatty acid composition and contents of lipid classes) and the occurrence and severity of focal lesions were both determined on the heart of each animal. Four groups were constituted according to the severity of cardiac lesions. Statistical analyses were applied to the data to find a relationship between the lipid parameters and the severity of heart lesions. None of the measured parameters (heart contents of neutral lipids, total phospholipids, phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, sphingomyelin and fatty acid composition of each phospholipid class) appeared to be related with the grading of the lesions. Therefore, we failed to find a direct support for the assumption that heart lesions, induced by LEAR oil, are mediated by changes in the lipid and/or fatty acid composition of heart membranes. However, this hypothesis can not be discarded.

rat — heart — lipids — fatty acids — phospholipids — necrosis — rapeseed oil

Résumé — Relations entre les paramètres lipidiques et l'apparition de lésions cardiaques et leur sévérité : étude chez des rats recevant de l'huile de colza pauvre en acide érucique. *Cinquante-six rats mâles Wistar EOPS pris au sevrage ont été nourris, pendant 18 semaines, avec un régime contenant 15% en poids d'huile de colza pauvre en acide érucique comme seule source de lipides alimentaires. Les analyses lipidiques (teneurs et composition en acides gras des classes de lipides) et les observations morphologiques (fréquence et sévérité des lésions) ont été faites sur le cœur de chaque animal. Quatre lots de rats ont été constitués sur la base du degré de sévérité des lésions cardiaques puis des analyses statistiques appropriées ont été appliquées aux données pour rechercher les relations qui pouvaient exister entre les paramètres lipidiques et la sévérité des lésions. Il apparaît qu'aucune des données biochimiques (teneurs du cœur en lipides totaux, en lipides non phosphorés totaux, en phospholipides totaux, en phosphatidylcholine, en phosphatidyl-éthanolamine, en disphosphatidylglycérol, en sphingomyéline et compositions en acides gras des classes phospholipidiques) ne peut être reliée au degré de sévérité des lésions. En conséquence, il*

* Correspondence and reprints.

n'a pas été possible de prouver que les lésions cardiaques induites par l'huile de colza pauvre en acide érucique étaient dues aux modifications de composition lipidique des membranes cardiaques. Toutefois, cette hypothèse ne peut pas être définitivement écartée.

rat — cœur — lipides — acides gras — phospholipides — lésions — huile de colza

Introduction

Several authors attempted to correlate lipid changes with the incidence or severity of lesions in hearts of rats fed various dietary fats. Gudbjarnason and Oskardottir (1975) reported that cod liver oil, as compared to a standard diet, induced significant changes in the polyunsaturated fatty acid composition of total heart phospholipids, namely an increased amount (x 1.7) of docosahexaenoic acid (22:6 n—3), and hypothesized that this rendered the organ more susceptible to necrosis. Beare-Rogers and Nera (1977) and Beare-Rogers *et al.* (1979) noticed that a 2 to 3-fold increase of 22:6 n—3 in cardiac phosphatidylcholine and phosphatidylethanolamine in rats fed low erucic acid rapeseed (LEAR) oil *vs* a fat mixture of lard and corn oil (3/1, w/w), coincided with an increased incidence of heart lesions. Trenholm *et al.* (1979), analyzing the data of several authors, concluded that the incidence of myocardial lesions in rats fed different oils, including LEAR oil, was positively correlated with the level of linolenic acid in the oils and negatively correlated with the level of saturated acids. Yamashiro and Clandinin (1980) associated the high level of n—9 fatty acids in heart mitochondrial phospholipids from rats fed high or low erucic acid rapeseed oils with the aggravated degenerative changes found in these organelles. Kramer (1980), Kramer and Sauer (1983), Kramer *et al.* (1985) confirmed that the incidence of heart lesions was reduced by increasing the level of saturated fatty acids in LEAR oil. They also tried to relate the amount of heart fatty acids (saturated and 22:6 n—3) derived from the dietary fatty acids with the incidence of heart lesions. However, in some of these studies (Trenholm *et al.*, 1979; Kramer *et al.*, 1985), even the use of elaborate statistical methods did not always lead to convincing conclusions. Moreover, Rocquelin *et al.* (1981) failed to clearly establish a relationship between the various changes in phospholipid contents or composition, and the incidence or number of lesions induced by sunflower, high- or low-erucic acid rapeseed oils in sedentary or trained rats.

The approach of the present study was somewhat different from the previous ones. Correlations between various lipid parameters and severity of heart lesions were not evaluated between groups of rats fed different diets but within a large single rat population being fed the same diet and developing different degrees of heart lesions. In order do so 56 male Wistar rats were fed LEAR oil as the only source of dietary fat. LEAR oil has a peculiar fatty acid composition (low concentration of saturates, high level of monoenes, high ratio of n—3/n— 6 (0.4— 0.5) polyunsaturated fatty acids) and rats constantly fed LEAR oil have had a higher incidence and/or severity of heart lesions, than rats fed control fats. Indeed, LEAR oil would be able to provoke more or less severe cardiac lesions in 11 to 100% of the fed animals, depending on the experimental conditions (Hulan *et al.*,

1977). At the end of the feeding period, both morphological examinations and complete lipid analyses were performed on the heart of each of the 56 rats. Rats were then allotted into 4 groups according to their degree of cardiac lesions, and lipid compositions were compared in between groups using appropriate statistical analysis to establish the relations. This procedure allowed us to determine if the individual heart sensitivity to the effects of LEAR oil was accompanied with some lipid changes.

Materials and Methods

Fifty-six weanling male (SPF) Wistar rats, weighing 77 ± 0.5 g, were housed in individual stainless steel cages. They were freely supplied with water and purified semi-liquid diets (Rocquelin *et al.*, 1986) containing refined LEAR oil 15% by weight. The main fatty acid composition (%) of LEAR oil was : 16:0, 5.0; 18:0, 1.6; 18:1 n—9, 51.2; 18:1 n—7, 5.7; 18:2 n—6, 23.9; 18:3 n—3, 8.6; 20:1 n—9, 1.6; 22:1 n—9, 1.1. At the end of 18 wk of feeding, the rats were sacrificed by decapitation after 8 h fasting. The hearts were removed, washed free from blood, weighed and transversally sliced in, 5 equal sections, with single edged razor blades fastened together. Three of the 5 sections (the auricular and the two apical portions) were collected and pooled for lipid analyses (0.7 g of fresh tissue). They were immediately stored in chloroform-methanol (2/l, V/V) containing 0.01% hydroquinone. The 2 intermediate sections were submitted to histological examination. One section was fixed in a Bouin solution, and then paraffin embedded. The other was soaked in isopentane, chilled in liquid nitrogen and sliced with a cryostatic microtome. In each case, 4—6 contiguous 5 µm slices were examined and severity of cardiac lesions was graded 0 to 4 as described in Grynberg *et al.* (1984).

Total heart lipids were extracted according to Folch *et al.* (1957). Phospholipids were separated from non-phosphorus lipids on silicic acid cartridges (Juanéda and Rocquelin, 1985). Quantification of the phospholipid classes was done as described previously (Rocquelin *et al.*, 1981). Phospholipid classes were separated by high performance liquid chromatography (Juanéda and Rocquelin, 1986), and their fatty acid composition was determined by gas liquid chromatography of the fatty acid methyl esters (Rocquelin *et al.*, 1981). Peaks were identified with the aid of known standard mixtures and quantified with a CDS 401 Varian integrator.

Three statistical approaches were applied to the collected data. As a first descriptive survey, correlation coefficients between histological grades, and other observed or calculated lipid parameters, were determined. Principal component analyses (PCA) were performed on 6 groups of the measured variables : heart lipid contents plus heart and body weights, fatty acid profiles of phosphatidyl-choline (PC), -ethanolamine (PE), diphosphatidylglycerol (DPG) and sphingomyelin (SPH) and a selection of 42 variables (see below). In each case, the histological grade was represented as an illustrative variable. Then a one-way analysis of variance (ANOVA) was performed on all variables, the factor being the histological grade. Rats graded 0 or 1 were grouped together and thus 4 groups of comparable size (12—16 animals in each) were constituted. This increased the robustness of the ANOVA with respect to normality and homogeneity of variances between groups (Dagnélie, 1975). Finally, a stepwise discriminant analysis was performed on the selection of 42 variables : body weight, heart weight, heart contents of non-phosphorus lipids, PC, PE, DPG, SPH and «other classes», all percentages of the 4 fatty acid profiles except the minor fatty acids (< 1%). This method determined the variables which best separated the 4 groups to yield a classification of the animals comparable to the real classification (% of «well-classed»). The multivariate analysis of variance Wilks' test was done with the aid of appropriate tables (Schatzoff, 1966; Pillai and Gupta, 1969; Lee, 1972). The discriminant analysis was first applied to the whole population ($n = 56$) then to a population from which 12 individuals were withdrawn (3 randomly chosen in each group) to constitute a test sample. This procedure was repeated with a second random test sample. The classification obtained for the test samples was an index of reliability of the discriminating space computed with the 2 (n—12) populations. Calculations were made on a Mini 6 CII Honeywell-Bull computer, using SPAD programs (Lebart and Morineau, 1982) for PCA, AMANCE programs (Bachacou *et al.*, 1981) for ANOVA and MAHA 3P program (Romeder, 1973) for stepwise discriminant analysis.

Results

Description of cardiac lesions and allotment of rats in 4 groups, according to the severity of lesions, are shown in Table I. Almost 25% of the examined hearts were considered as normal or subnormal (group I) whereas ≈ 75% of the rats had more or less severe lesions (groups II to IV). The proportion of hearts with lesions coincided with previous observations (Kramer *et al.*, 1975; Beare-Rogers and Nera, 1977; Beare-Rogers *et al.*, 1979; Rocquelin *et al.*, 1981; Kramer *et al.*, 1982).

Body and organ weights, heart lipid contents and fatty acid profiles of phospholipid classes in the 4 groups, are shown in Tables II-VI. The percent of fatty acid composition of heart phospholipids reflected the nature of ingested dietary fat (see Stubbs and Smith, 1984 and McMurchie, 1988, for reviews). The presence of linolenic acid in LEAR oil had a strong effect on polyunsaturated fatty acid profiles of heart PC and PE. The 22:5 and 22:6 n—3 were greatly increased at the expense of 22:4 and 22:5 n—6, which disappeared almost completely. Our results show a remarkable similarity with those obtained previously (Beare-Rogers *et al.*, 1979; Kramer, 1980; Rocquelin *et al.*, 1981). For instance, 22:6 n—3 in PE represents here nearly 20% of the total fatty acids, which is within the range of 17.6 to 22.8% obtained in the above-mentioned studies, a 2—6-fold increase; this is compared to control rats fed various fats which are low in linolenic acid content.

In this study, correlation coefficients between histological grades and other lipid parameters were low : from —0.25 to + 0.31. PCA did not reveal any relation between histological grades and other variables. ANOVA confirmed this result : none of the Fischer's F—tests were significant at the 5% level.

The stepwise discriminant analysis (SDA) performed on the whole population, (n=56) gave a significant discrimination, but only after the input of many variables :

Table I. Division of rats according to the severity of cardiac lesions (n = 56)

Group	*Grade of severity*	N	*Pathological observations*
I	0 to 1	14	Normal tissue, but in some cases dilatation of microvessels and hyaline interfibrillar depositions
II	2	12	Foci of hyperacidophilia and/or muscular degeneration Dilatation of microvessels
III	3	16	More or less pronounced foci of necrosis associated with loose fibrosis and sparse mesenchymal cell infiltration
IV	4	14	Completly necrotized foci filled with a close fibrosis or dense mesenchymal cell infiltration

Table II. Body, heart, cardiac lipid and phospholipid class weights of rats in the different morphological groups [a].

	I n = *14*	*II* n = *12*	*III* n = *16*	*IV* n = *14*	*SEM*
Body weight (g)	577	571	600	586	11.2
Heart weight (g)	1.15	1.20	1.30	1.23	0.06
Total lipids [b]	38.6	46.6	45.7	42.5	3.06
Non phosphorus lipids	14.9	22.7	21.6	17.7	3.02
Total phospholipids	23.7	23.9	24.1	24.8	0.55
Phosphatidylcholine	9.6	10.0	10.2	10.8	0.32
Phosphatidyletanolamine	7.7	8.0	8.2	8.5	0.43
Diphosphatidylglycerol	3.7	3.5	3.2	3.5	0.32
Sphingomyelin	0.6	0.4	0.5	0.5	0.06
Other classes [c]	2.0	2.0	2.0	2.6	0.22

[a] See Table I for group definition.
[b] Lipid contents are expressed as mg/g of wet tissue. SEM is the standard error of the mean calculated for $n = 14$.
[c] Phosphatidylinositol + phosphatidylserine + lysophosphatidylcholine.

27 variables to reach 91% of well-classed individuals. When performed on the (n—12) populations, SDA led to somewhat different discriminating factors, and the 2 test samples were not "well-classed". The significance of the discriminating space appears, therefore, to be fragile. Moreover, the fact that the 4 groups were constituted *a posteriori* and not *a priori*, required a conservative attitude when interpretating the results of SDA. In conclusion, the present data show no significant and reliable correlation between the severity of heart lesions and any of the lipid variables.

Discussion

The fact that heart lesions, observed in LEAR oil-fed rats, are due to the fatty acid composition of the oil, is supported by strong experimental evidence. Kramer *et al.* (1975), investigating the cardiopathogenicity of several fractions obtained by adsorption chromatography or molecular distillation of a LEAR oil, concluded that if some factor other than erucic acid was responsible for the cardiotoxicity of the oil, it was not in the unsaponifiable matter, but rather in the triglyceride fraction. Moreover, hardening of LEAR oil (Beare-Rogers *et al.*, 1974), or supplementing the oil with saturated fatty acids (Farnworth *et al.*, 1982; Kramer *et al.*, 1982; Clandinin and Yamashiro, 1983), decreased the incidence and/or severity of heart lesions.

The specific role of 18:3 n—3 of LEAR oil in the etiology of cardiac necrosis was postulated by McCutcheon *et al.* (1976) and Trenholm *et al.* (1979). However, the cardiopathogenic effect of 18:3 n—3 was not clearly distinguished from a global LEAR oil effect.

Dietary fats greatly influence the phospholipid composition of membranes,

Table III. Fatty acid composition of heart phosphatidylcholine [a].

Main fatty acids [c]	*I* [b]	*II*	*III*	*IV*	*SEM*
	n = *14*	n = *12*	n = *16*	n = *14*	
16:0 DMA[d]	0.6	0.6	0.6	0.6	0.04
16:0	15.0	14.8	15.4	15.5	0.38
16:1 n–9	0.2	0.2	0.2	0.2	0.03
18:0	26.6	26.6	26.3	26.7	0.42
18:1 n–9	6.8	7.0	7.2	6.9	0.17
18:1 n–7	5.2	5.1	5.4	5.3	0.12
18:2 n– 6	11.3	11.5	11.9	10.8	0.39
18:3 n–3	0.3	0.3	0.3	0.3	0.02
20:2 n– 6	0.3	0.3	0.3	0.3	0.02
20:3 n– 6	0.5	0.5	0.5	0.5	0.05
20:4 n– 6	25.0	25.2	24.6	25.3	0.41
22:5 n–3	2.5	2.4	2.3	2.4	0.14
22:6 n–3	3.8	3.6	3.4	3.5	0.21
Double bond index [e]	1.7	1.7	1.7	1.7	0.02
Σ saturates	41.6	41.4	41.7	42.2	0.35
Σ monounsaturates	12.2	12.2	12.9	12.4	0.25
Σ polyunsaturates	43.7	43.9	43.1	43.0	0.35
Σ n–9	7.0	7.2	7.4	7.1	0.17
Σ n– 6	37.0	37.6	37.2	36.9	0.29
Σ n–3	6.6	6.3	5.9	6.2	0.32
n– 6/n–3	5.9	6.1	6.4	6.1	0.26
18:2 n– 6/22:6 n–3	3.2	3.3	3.7	3.2	0.24
20:4 n– 6/22:6 n–3	6.9	7.3	7.5	7.4	0.33
20:4 n– 6/18:2 n– 6	2.2	2.2	2.1	2.4	0.11
Polyunsat./sat.	0.16	0.15	0.14	0.15	0.005

[a] Values shown are % total fatty acids. SEM is the standard error of the mean calculated for $n = 14$.

[b] See Table I for group definition.

[c] Minor amounts of 16:1 n–7, 17:0, 18:0 DMA, 18:1 DMA, 20:0, 20:1 n–9, 20:5 n–3, 22:4 n–6, 22:5 n–6 were detected, but are not shown in the Table.

[d] DMA = dimethylacetals derived from alkenylethers during methylation.

[e] Double bond index is the sum of % of individual unsaturated fatty acids x number of double bonds/100.

especially in the heart (see McMurchie, 1988, for a review). These changes are suspected to render the membrane more or less fragile or permeable, which could lead to cardiac pathology or disfunction (Yamashiro and Clandinin, 1980; McMurchie *et al.*, 1983; Rocquelin *et al.*, 1986; McMurchie, 1988). Preliminary results in our laboratory (Grynberg *et al.*, 1984), showed that cardiac mitochondrial succinate dehydrogenase activity (demonstrable by histological methods) increased with the severity of the lesions, observed in hearts similar to those used in the study. Nevertheless, it was not possible to relate the occurence and severity of lesions, with any changes of the measured lipid parameters, from the results of this study. Although a great variation in the severity of heart lesions

Table IV. Fatty acid composition of heart phophatidylethanolamine [a].

Main fatty acids [b]	*I*	*II*	*III*	*IV*	*SEM*
	n = *14*	n = *12*	n = *16*	n = *14*	
16:0 DMA	4.3	4.5	4.6	4.7	0.16
16:0	5.5	5.7	5.9	5.9	0.27
18:0 DMA	2.7	2.7	3.0	3.3	0.24
18:1 DMA	4.8	4.9	4.7	4.6	0.18
18:0	21.5	21.2	21.3	21.8	0.33
18:1 n–9	6.2	6.6	6.4	6.2	0.19
18:1 n–7	3.2	3.3	3.4	3.3	0.20
18:2 n– 6	4.8	5.0	4.8	4.8	0.24
20:4 n– 6	19.3	19.7	19.4	18.5	0.42
22:5 n–3	4.7	4.6	4.6	4.7	0.19
22:6 n–3	20.4	18.9	19.2	19.4	0.78
Double bond index	2.5	2.4	2.4	2.4	0.05
Σ saturates	27.0	26.9	27.2	27.7	0.51
Σ monounsaturates	9.4	9.8	9.8	9.5	0.35
Σ polyunsaturates	49.1	48.2	48.0	47.3	0.83
Σ DMA	11.8	12.2	12.2	12.6	0.42
Σ n– 6	24.0	24.7	24.2	23.2	0.56
Σ n–3	25.1	23.4	23.8	24.1	0.84
n–6 / n–3	1.0	1.1	1.0	1.0	0.06
18:2 n– 6 / 22:6 n–3	0.2	0.3	0.3	0.3	0.02
20:4 n– 6 / 22:6 n–3	1.0	1.1	1.1	1.0	0.06
20:4 n– 6 / 18:2 n– 6	4.1	4.0	4.2	4.0	0.20
Polyunsat./Sat.	1.8	1.8	1.8	1.7	0.06

[a] See Table III for definitions.

[b] Minor amounts of 16:1 n–9, 16:1 n–7, 17:0, 18:3 n–3, 20:0, 20:1 n–9, 20:2 n–6, 20:3 n–6, 20:5 n–3, 22:4 n-6, 22:5 n–6 were detected, but are not shown in the Table.

was observed, the lipid compositions remained in a narrow spread. Membrane lipid profiles reflect many metabolic ways (desaturation, elongation, esterification etc.), but nevertheless, there was no support for an involvement of individual susceptibility, which was the case in the development of lesions. This does not mean that the hypothesis of the cardiopathogenic effect of LEAR oil, due to membrane lipid composition, must be discarded. The fatty acid alterations induced by LEAR oil in heart membrane phospholipids can make cell membranes more susceptible to the onset of lesions, but perhaps the pathological process is initiated individually by some unknown factor. This explains why, in the same population of rats fed LEAR oil, certain individuals had lesions whilst others did not. According to this assumption, the heart lipid changes induced by dietary fats can be related to a frequency of lesions in one population, but not to the individual severity of lesions. The alternate hypothesis would be that the cardiopathogenic effect of LEAR is not mediated by membrane lipid or fatty acid changes, but

Table V. Fatty acid composition of heart diphosphatidylglycerol [a].

Main fatty acids [b]	*I*	*II*	*III*	*IV*	*SEM*
	n = *14*	n = *12*	n = *16*	n = *14*	
16:0 DMA	0.3	0.3	0.3	0.4	0.07
16:0	2.9	2.9	2.8	2.9	0.17
16:1 n–9	0.6	0.7	0.7	0.7	0.12
16:1 n–7	0.8	0.8	0.8	0.8	0.07
18:0	2.4	2.2	2.3	2.4	0.13
18:1 n–9	5.3	5.9	5.8	5.8	0.29
18:1 n–7	3.5	4.0	4.0	4.1	0.26
18:2 n– 6	75.5	75.0	74.9	75.0	0.94
20:2 n– 6	0.7	0.7	0.8	0.7	0.05
20:3 n– 6	1.3	1.2	1.2	1.3	0.08
20:4 n– 6	2.8	2.7	2.7	2.5	0.17
22:6 n–3	2.5	2.1	2.4	2.1	0.22
Double bond index	1.9	1.9	1.9	1.9	0.01
Σ saturates	5.2	5.1	5.1	5.3	0.22
Σ monounsaturates	10.3	11.4	11.3	11.4	0.57
Σ polyunsaturates	82.8	81.7	82.1	81.6	0.75
Σ n–9	6.0	6.6	6.5	6.5	0.33
Σ n–7	4.3	4.8	4.8	4.9	0.28
Σ n– 6	80.3	79.6	79.6	79.5	0.82
n– 6 / n–3	32.1	43.6	38.7	42.1	4.29
18:2 n– 6 / 22:6 n–3	30.2	41.1	36.4	39.8	4.11
20:4 n– 6 / 22:6 n–3	1.1	1.4	1.3	1.2	0.12
20:4 n– 6 / 18:2 n–6	0.04	0.04	0.04	0.03	0.003
Polyunsat./Sat.	15.9	16.6	16.4	15.7	0.76

[a] See Table III for definitions.

[b] Minor amounts of 17:0, 18:0 DMA, 18:1 DMA, 20:0, 20:1 n–9, 22:5 n–3 were detected, but are not shown in the Table.

would imply some other mechanism. To date, there is no clear experimental evidence to support such a hypothesis.

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Table VI. Fatty acid composition of heart sphingomyelin [a].

Fatty acids	*I*	*II*	*III*	*IV*	*SEM*
	n = 14	*n = 12*	*n = 16*	*n = 14*	
16:0	14.6	15.0	16.3	16.3	0.58
16:1 n–9 + n–7	1.0	1.1	1.2	1.2	0.20
17:0	0.8	0.6	0.7	0.7	0.10
18:0	24.1	23.8	24.1	25.1	0.93
18:1 n–9	3.7	3.5	3.6	3.8	0.42
18:1 n–7	0.9	0.8	0.9	0.8	0.11
18:2 n– 6	0.8	0.7	0.9	0.7	0.09
20:0	19.9	20.2	19.6	20.2	0.66
22:0	18.6	18.6	17.4	17.9	0.56
24:0	9.4	9.2	9.2	8.5	0.44
24:1 n–9	6.2	6.4	6.0	4.8	0.64
Double bond index	0.1	0.1	0.1	0.1	0.01
Σ saturates	87.3	87.4	87.3	88.6	1.00
Σ monounsaturates	11.9	11.9	11.7	10.7	0.98
Monounsat./sat.	0.1	0.1	0.1	0.1	0.01

[a] See Table III for definitions.

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