Iodine-125-fibrinogen kinetics in the rabbit arterial wall

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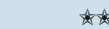

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Abstract

Elevated fibrinogen has been claimed as an independent risk factor for the development of atherosclerosis. Incorporation of fibrinogen into human atherosclerotic lesions has been demonstrated. *We assessed* in a rabbit model of experimental atherosclerosis, biodistribution as well as kinetics and vascular uptake of ^{125}I-fibrinogen. *Rabbits aged* 6 months were fed a 1% cholesterol supplemented diet. After experimental de-endothelialization of rabbit aorta using a Fogarthy catheter, ^{125}I-fibrinogen uptake was enhanced by more than one order of magnitude as compared to intact segments covered by endothelium. Six rabbits per group were examined. *Even re-endothelialized segments showed* a significantly higher uptake of the radiolabeled protein. Maximum arterial uptake varied between 12 (de- and re-endothelialized segments) and 24h (intact areas) after injection of ^{125}I-fibrinogen. *In conclusion,* these experiments for the first time suggest the increased uptake of radiolabeled fibrinogen in the aortic de-endothelized wall in rabbits.

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Introduction

In prospective studies, fibrinogen has been demonstrated to be independently associated with arterial events [1]. In particular, a correlation of elevated plasma fibrinogen with coronary heart disease [2-4] was reported. The incrustation theory of the Viennese pathologist Carl von Rokitansky that may be the first description of arterial fibrinogen deposition, dates back to 1852 [5]. The incorporation of fibrinogen into human atherosclerotic lesions has been described in detail by J. Duguid 1946 [6]. Repeated thrombosis and organization may produce accumulation of fibrous tissue becoming incorporated into the arterial wall. Fibrinogen as an acute phase reactant highly correlated with other cardiovascular risk factors like smoking, diabetes, age, etc. may favor arterial thrombotic disease via several of mechanisms, including cellular proliferation, viscosity, platelet aggregation and fibrin formation [7]. A correlation between high procoagulant factors, fibrinogen and persistent thrombin generation and restenosis has been documented [8]. Interestingly, radiolabeled fibrinogen was used very rarely for the study of human and experimental atherosclerotic lesions. Application of differently radiolabeled (^{123}I, ^{125}I or ^{131}I)- fibrinogen using a variety of methodologies [9] has been performed to image single cases of peripheral vascular disease in the femoral and carotid arteries [10] as well as in myocardial infarction, in men [11, 12]. A series of data by Born´s group described the use of tyramine cellobiose for ^{131}I-fibrinogen labeling [13-18]. Experimental data with intact native fibrinogen and in particular human findings assessing the uptake and the kinetics of radiolabeled fibrinogen in-vivo are so far lacking. Furthermore, the potential problem of viral contamination such as in hepatitis or in auto immune diseases restricted its use to autologous protein, its separation and radiolabeling being too complicated and time consuming for clinical routine. We therefore examined the arterial uptake of native ^{125}I-fibrinogen in a well known and established rabbit model of experimental atherosclerosis [19] to assess whether elevated cholesterol might enhance vascular uptake of fibrinogen.

Materials and methods

Animal experiments

After the abrasion of the abdominal aorta using a Fogarthy catheter as described earlier [20], six male New Zealand white rabbits aged 6 months were fed for 4 weeks a 1% cholesterol supplemented diet. Six non-cholesterol fed animals served as controls. Iodine-125-fibrino-

gen was injected via the ear vein. Animals were killed at different time intervals (1, 3, 6, 12 and 24h) after tracer injection. Vascular segments and organ tissues were excised and fixed in phosphate-buffered (pH 7.4) glutaraldehyde. Counting was performed to assess biodistribution and expressed as % of total radioactivity and vascular uptake after characterization of surface lining. The morphology of surface lining was assessed by light microscopy in paraffin-embedded sections (5μ) stained by haematoxylin/eosin and van Giesson. Semithin-sections stained by toluidine blue were used to assess surface lining.

Fibrinogen-labeling

Autologous rabbit fibrinogen was isolated as described by us [21] and radiolabeled with ^{125}I by chloramine-T [22]. Protein modification was excluded. Quality control was performed using thinlayer-chromatography and cellulose acetate electrophoresis as well as functional testing.

Plasma clearance

Decay was assessed by drawing blood samples at different time intervals (0, 30 and 45min, as well as 1, 3, 6, 12 and 24h) after injection of ^{125}I-fibrinogen from those animals being killed at the 24h interval and subsequent counting. The 0min value served as 100%. Furthermore, % radioactivity of ^{125}I still bound to fibrinogen was assessed at the identical time intervals.

Statistical analysis

Values are presented as x ± SD; calculation for significance was done using ANOVA.

Results

The biodistribution of ^{125}I-fibrinogen is given in Table 1. The radiolabeled protein is found mainly in the lung and the liver. Hypercholesterolemic (HC) animals retain much more fibrinogen in the arterial wall as do control normocholesterolemic (NC) animals. For the other organs there is no difference in biodistribution between HC and NC. The kinetics of arterial uptake show a clear correlation to the type of surface lining. In de- and re-endothelialized segments maximum uptake in the arterial wall is seen already at 12h after injection of the radiolabeled protein; in intact endothelialized areas, however, at about 24h or later. At all time intervals, in intact areas the uptake was by far the lowest and in de-endothelialized ones the highest (Table 2). At certain lesion sites, the ^{125}I-fibrinogen uptake was even up to 100-fold increased. Fibrinogen half life in NC animals was 4.84 ± 0.27 days, in HC 4.69 ± 0.31, the difference not reaching significance. Even 24h after injection of radiolabeled fibrinogen, the majority of the label (92.7±3.18%) was still fibrinogen bound. Quality control of fibrinogen revealed no significant functional changes after radiolabeling (Table 3). Blood disappearance did not differ significantly between HC and NC throughout the entire observation period (data not shown).

Discussion

Fibrinogen is a soluble protein secreted into plasma from hepatocytes coregulating hemostasis. Its synthesis is increased during inflammatory processes thus serving as a marker for atherogenicity. The vascular uptake of ^{125}I-fibrinogen is 2-3 times higher as compared to the one found in a similar experi-

Table 1. *Biodistribution of ^{125}I-fibrinogen in rabbits*

	1 h	3 h	6 h	
Liver	20.17 ± 1.12	17.90 ± 3.16	18.20 ± 4.02	HC
	19.36 ± 1.127	18.03 ± 3.27	18.10 ± 3.77	NC
Spleen	1.06 ± 0.08	1.04 ± 0.09	1.21 ± 0.11	HC
	1.03 ± 0.07	1.08 ± 0.06	1.19 ± 0.09	NC
Kidney	6.21 ± 0.84	5.97 ± 0.93	5.86 ± 0.80	HC
	6.16 ± 1.12	6.08 ± 0.87	6.01 ± 0.77	NC
Heart	3.89 ± 0.46	5.41 ± 0.67	6.07 ± 0.84	HC
	3.75 ± 0.44	5.20 ± 0.71	5.88 ± 0.75	NC
Lung	31.07 ± 5.21	29.46 ± 6.04	29.88 ± 5.71	HC
	31.44 ± 4.86	30.80 ± 5.19	30.07 ± 4.88	NC
Stomach	0.65 ± 0.08	0.69 ± 0.11	0.75 ± 0.07	HC
	0.67 ± 0.05	0.69 ± 0.10	0.74 ± 0.08	NC
Intestine	3.56 ± 0.19	3.91 ± 0.47	4.27 ± 0.53	HC
	3.66 ± 0.22	3.80 ± 0.56	4.43 ± 0.67	NC
Intact Aorta	0.51 ± 0.09	0.64 ± 0.12	0.84 ± 0.13	HC
	0.37 ± 0.07*)	0.43 ± 0.09*)	0.60 ± 0.67	NC
Adrenal	4.16 ± 0.39	4.27 ± 0.46	4.44 ± 0.50	HC
	4.57 ± 0.50	4.86 ± 0.59	4.77 ± 0.63	NC

Data in % of injected activity; x ± SD; n = 6 each; *) P < 0.01 (HC vs. NC)
HC: hypercholesterolemic; NC: normocholesterolemic

Table 2. *Fibrinogen kinetics in endothelialized (E), de-endothelialized (D) and re-endothelialized (R) segments*

	1 h	3 h	6 h	12 h	24 h	
E	451 ± 143	605 ± 143	924 ± 267	2256 ± 205	2635 ± 361	HC
	364 ± 132	427 ± 156*	731 ± 243	1663 ± 246*	2107 ± 280	NC
D	4398 ± 596	7106 ± 1513	19216 ± 2419	27381 ± 5114	24265 ± 4766	HC
	3586 ± 471	5396 ± 1206*	12306 ± 1486*	17493 ± 3766*	15987 ± 3299*	NC
R	956 ± 241	2566 ± 312	3108 ± 421	5641 ± 1216	5410 ± 1408	HC
	716 ± 204	2034 ± 227*	2617 ± 330	4308 ± 984*	3809 ± 1116*	NC

Values in cpm/g tissue; x ± SD; n = 6 each; *) P < 0.01
(NC: normocholesterolemic vs HC: hypercholesterolemic)

Table 3. *Tracer binding to fibrinogen (% of injected activity)*

Time	0 min	30 min	1 h	3 h	6 h	12 h	24 h
NC	100	99.7±0.91	98.3±1.21	97.6±1.16	95.8±1.94	94.7±2.06	92.2±3.18
HC	100	99.8±0.86	99.1±0.94	97.7±1.71	95.3±1.56	94.2±2.16	93.3±2.95

Mean values of 6 experiments each (± SD); NC: normocholesterolemic;
HC: hypercholesterolemic

ment for LDL [9, 19]. It has been shown that using tyramine cellobiose labeled fibrinogen the application of a variety of pressor agents over a few days, fibrinogen and LDL uptake by the arteries was accelerated. The total uptake by the arteries**, however,** was not faster as in rats genetically hypertensive for about 3 months [13].

Angiotensin II (A II) at different concentrations increased human fibrinogen uptake in rat aorta independent of the dose [14]. In spontaneously hypertensive rats, where blood pressure was even higher, enhanced fibrinogen uptake was not monitored. The authors thus concluded that the effect may be independent from the pressor action. Angiotensin II (25nM) and adrenaline (10nM) were shown to increase arterial fibrinogen uptake by 109% and 31%, respectively [16]. In contrast, desoxycorticosterone acetate administration-although increasing arterial blood pressure-did not alter arterial fibrinogen accumulation [12]. Treating normotensive and spontaneously hypertensive Wistar rats with L-NAME, an inhibitor of nitric oxide, did not alter the fibrinogen-nor the LDL-uptake in the aortic wall [15]. Neither captopril, an angiotensin converting enzyme inhibitor, nor losartan, an A II-receptor antagonist were able to influence fibrinogen uptake in normo- as well as hypertensive rats [18]. All these studies were performed using human fibrinogen in rats. In contrast, our results in rabbits were obtained using autologous rabbit fibrinogen. Eventual differences between auto-, homo- and heterologous protein have not been communicated so far, especially concerning turnover rate, halflife and catabolism. Data on autologous radiolabeled fibrinogen in rabbits and rats are not available. It is thus of interest to consider that rat and human LDL investigated in rats exhibited different results [15]. Considering size and density distribution of fibrinogen, this difference becomes understandable. In all the above mentioned studies, the adduct tyramine cellobiose was used and the protein was labeled with ^{125}I or ^{131}I. Modification of proteins may play a central role in atherogenesis. Extent and functional consequence of oxidative [23], glycated [24], acetylated [25], nitrated [26], glycoxidated and other modifications of fibrinogen and the potential influence of drugs (vitamins) are still largely unknown. Commercially available fibrinogen from various companies revealed a greatly varying extent of oxidative modification [27]. No data on structural modification of fibrinogen, either induced by isolation, separation and labeling (e.g. oxidation), or molecular heterogeneity or intentional use of certain well defined modified molecular forms are available. Failure of radiolabeled fibrinogen to detect thrombi in men may be due to molecular modification [28] and consequently altered function of behaviour as well as the use of homologous protein. These aspects as well as the problem of viral contamination terminated its use for radionuclide imaging. A comparison of glycated (about 6% of total fibrinogen) vs. non-glycated fibrinogen in rabbits revealed no significant difference in its half life [29]. On the other hand, reduced fibrinogen survival in diabetics was reported [30]. Dunn et al. 2005 reported in type II diabetics a denser, less porous structure of fibrinogen related to glycemic control [31]. Acetylation has been found to result in a looser fibrin and to render it less resistant to fibrinolysis [32]. No quality control as to an accidental protein modification is available. The excellent recovery and kinetics of ^{125}I-fibrinogen (Table 3) indicates no significant damage to the radiolabeled protein by the labeling technique. The stability data exclude a significant breakdown of radiolabel and reveal that the accumulation of radioactivity in the vessel wall is in fact due to ^{125}I-fibrinogen.

Endothelial damage induces a haemostatic response including platelet deposition and activation of coagulation cascade and finally fibrinogen adsorption at the site of injury [29] depending on local thrombin concentrations. In humans, ^{123}I-fibrinogen uptake revealed that atherosclerotic lesion sites can be identified best 4h after injection and to a lesser extent after 24h, while interestingly in our experimentally damaged vascular segments, 12h was the interval exhibiting maximum arterial uptake [33]. Reasons for this difference are unknown. Our earlier human studies did not reveal a correlation between local uptake of ^{123}I-fibrinogen and sonography. A maximum of fibrinogen accumulation in the aorta of diabetic rabbits was seen at 12h after injection with an increased fibrinogen/antithrombin III ratio characteristic for the prothrombin state [34]. The prevalence of lesions in the lower extremities in men was much higher as compared to the carotid arteries [33]. For reasons of size and methodological aspects, we were using the aorta in the experimental setting. Interestingly, sites of enhanced uptake of fibrinogen and LDL turned out to be identical [9]. Furthermore, concerning the arterial uptake kinetics, there are some similarities between these proteins. Although both hypercholesterolemia as well as experimental lesioning are increasing vascular uptake, the amount of fibrinogen trapped is insufficient in order to cause significant shortening of decay of the protein in the plasma.

The investigation of the behaviour of structurally different fibrinogen molecules (oxidation, glycation, etc) will further contribute to the understanding of atherosclerotic lesion formation. The need to use autologous protein and the difficult and time consuming isolation of fibrinogen, however, will preclude a comeback of radiolabeled fibrinogen for routine diagnosis of thrombosis in men.

In conclusion, our findings for the first time in vivo show that arterial surface lining and hypercholesterolemia are relevant determinants for local fibrinogen uptake and also affect local and as understood above, systemic fibrinogen kinetics.

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