Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study

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Summary

Indoleamine 2,3 dioxygenase (IDO), an enzyme involved in the catabolism of tryptophan, suppresses T cell activity and is up-regulated by various inflammatory stimuli. The ratio of kynurenine, the main metabolite of tryptophan, to tryptophan (kyn/trp) reflects IDO activity. We calculated IDO activity and measured carotid intima-media thickness (IMT), a presymptomatic predictor of atherosclerosis, in 986 young adults (544 female, 442 male) for whom data on levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, high sensitive C-reactive protein (CRP), body mass index (BMI), waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure and smoking habits were available. IDO activity correlated significantly with IMT in female subjects, but not in males. In a multivariate linear regression model, IDO did not correlate independently with IMT in female subjects. However, IDO activity correlated significantly with several risk factors for atherosclerosis in females, i.e. with age, LDL-C, BMI, weakly with CRP and inversely with HDL-C and triglyceride. In males IDO activity correlated significantly with CRP and inversely with HDL-C. In conclusion, our results suggest that the IDO enzyme is involved in the immune regulation of early atherosclerosis, particularly in young female adults, and could constitute a novel marker of immune activation in early atherosclerosis in females.

Keywords: atherosclerosis, indoleamine 2,3-dioxygenase, inflammation

Introduction

The inflammatory nature of atherosclerosis is currently well recognized (for reviews, see [1–3]). The atherosclerotic plaque contains inflammatory cells such as macrophages and T lymphocytes, most of which are of the Th1 phenotype, i.e. proinflammatory cells. The plaque macrophages take up oxidized low-density lipoprotein (LDL) or are activated by microbial components or autoantigens (e.g. heat shock proteins), leading to production of proinflammatory cytokines and presentation of the antigenic material to the Th1 cells. Thereafter the macrophages differentiate gradually to lipid-loaded foam cells, i.e. activated macrophages. This chain of events resembles a normal immune response. The normal immune/inflammatory response induced by an antigen is followed by activation of several inhibitory mechanisms, which is also the case in the immune response associated with atherosclerosis. The immunoregulatory cytokines interleukin (IL)-10 and transforming growth factor (TGF)-β1 have been associated with protection from atherosclerosis in mouse models [4,5] and in human disease [6].

Recently, indoleamine 2,3-dioxygenase (IDO) has drawn considerable attention as another mechanism of immune regulation [7–10]. IDO is an enzyme involved in the catabolism of the essential amino acid tryptophan, and the ratio of kynurenine, the main metabolite of tryptophan, to

tryptophan (kyn/trp) can be used to reflect IDO activity. IDO is expressed mainly in antigen-presenting cells (APCs), i.e. at the initiation of the immune response. Its activation leads to a decrease in the tryptophan concentration in local microenvironments, thus suppressing the activation of surrounding T lymphocytes ('suppression by starvation') [8].

T cells in atherosclerotic plaques are mainly of the Th1 subtype, secreting, e.g. the proinflammatory cytokine interferon (IFN)-γ [2]. IDO is up-regulated in response to various infectious and inflammatory stimuli and IFN-γ, a Th1 cytokine, is also a strong inducer of IDO. Our aim here was to test the hypothesis that IDO is involved in the regulation of inflammatory responses associated with the development of early atherosclerosis. To this end we investigated the activity of IDO, i.e. kyn/trp, and measured carotid intima-media thickness (IMT), a presymptomatic predictor of atherosclerosis, as well as traditional risk factors for atherosclerosis in 986 (544 female, 422 male) young adults.

Materials and methods

Subjects

The Cardiovascular Risk in Young Finns Study is a prospective multi-centre cohort study being conducted in five university hospital cities in Finland; it was initiated in 1980, with the latest control visits made and laboratory data gathered in 2001. The cohort in the current study comprised 986 participants (544 female, 442 male aged 24–39 years) from two of the centres, the cities of Helsinki and Turku and their rural surroundings.

The data on cardiovascular risk variables in the cohort in 2001 (levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, body mass index (BMI), waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure, smoking habits) had been recorded by methods described previously [11]. Plasma high sensitive C-reactive protein (CRP) concentrations had been analysed by latex turbidometric immunoassay (Wako Chemicals GmbH). Carotid IMT measurements were performed in 2001, as described elsewhere [11].

Tryptophan and kynurenine determinations

Tryptophan (μmol/l) and kynurenine (μmol/l) concentrations in peripheral blood were measured by reverse-phase high-performance liquid chromatography (HPLC), as described previously [12]. Tryptophan was separated with a Shimadzu liquid chromatograph LC-10AD VP (Shimadzu Co, Kyoto, Japan) using a 50-mm BDS Hypersil C18 5 μm column (Thermo Electron Co, Bellefonte, PA, USA). It was monitored by fluorescence with a Shimadzu RF-10A XL detector at 266 nm excitation and 366 nm emission wavelengths. Kynurenine was separated with a Hewlett

Packard 1100 liquid chromatograph (Palo Alto, CA, USA) using Merck LiChroCart 55–4150 mm cartridge containing a Purospher STAR RP-18 3 μm column (Merck Co, Darmstadt, Germany). It was determined by ultraviolet absorption at 360 nm wavelength with a Hewlett Packard G13144 detector. Kyn/trp (μmol/mmol) was calculated by relating concentrations of kyn (μmol/l) to trp (mmol/l), this allowing an estimate of IDO activity.

Statistical analysis

Values for CRP and triglycerides were transformed logarithmically prior to statistical analyses due to their skewed distributions. Smoking was regarded as a dichotomous variable (current and ex-smokers *versus* never-smokers). Comparisons of continuous and dichotomous variables were performed by Student's *t*-test and χ^2 test, respectively. Correlation was calculated with the Pearson correlation coefficient. Multivariate stepwise linear regression analysis was performed to analyse the independent effect of kyn/trp on IMT. Findings were considered statistically significant at $P < 0.05$. Statistical analyses were performed with SPSS version 13·0 for Windows.

Ethical considerations

The study was approved by the local ethics committees and all subjects participating in the study gave written informed consent.

Results

The data on various risk factors for atherosclerosis in female and male subjects, as well as tryptophan and kynurenine concentrations and kyn/trp, are presented in Table 1.

The mean ($\pm$ s.d.) kyn/trp was 27.4 ± 7.2 μmol/mmol in all subjects, 27.0 ± 7.2 μmol/mmol in females and 27.9 ± 7.3 μmol/mmol in males. Kyn/trp correlated weakly with mean IMT in all subjects ($r = 0.091$, Pearson's correlation coefficient, $P = 0.004$). In females the correlation was more pronounced ($r = 0.131$, $P = 0.002$), while in men kyn/trp did not correlate with IMT ($r = 0.040$, $P = 0.402$). The correlation coefficients between kyn/trp and the risk variables for atherosclerosis in female and male subjects are shown in Tables 2 and 3, respectively.

In addition to IDO activity, age, BMI and systolic blood pressure proved significant predictors of IMT in univariate analysis in females (Table 4). To assess the independent effect of kyn/trp on IMT in females, a multivariate linear regression model was constructed with IMT as the dependent variable and the traditional cardiovascular risk factors (age, HDL-C and LDL-C, logarithmically transformed concentration of triglyceride, BMI, systolic and diastolic blood pressure, smoking) and the logarithmically transformed

Table 1. Risk factors for atherosclerosis, tryptophan (trp) and kynurenine (kyn) concentrations and kyn/trp ratio in 986 young (24–39 years) adults.

Variable	Females, $n = 544$	Males, $n = 442$
Age, years	31·5 ± 4·9	31·9 ± 5·0
HDL, mmol/l	1·40 ± 0·30	1·13 ± 0·28*
LDL, mmol/l	3·11 ± 0·76	3·45 ± 0·91*
Triglyceride, mmol/l	1·15 ± 0·58	1·55 ± 1·09*
BMI, kg/m^2	24·0 ± 4·3, $n = 525$	25·7 ± 3·9*, $n = 433$
Waist circumference, cm	78·0 ± 11·1, $n = 531$	89·3 ± 10·8*, $n = 441$
Waist-to-hip ratio	0·78 ± 0·06, $n = 529$	0·89 ± 0·06*, $n = 441$
Systolic blood pressure, mmHg	115 ± 13, $n = 541$	129 ± 13*, $n = 438$
Diastolic blood pressure, mmHg	71 ± 9, $n = 541$	75 ± 9*, $n = 438$
CRP, mg/l	2·43 ± 5·11	1·61 ± 0·60**
IMT, mm	0·57 ± 0·08, $n = 541$	0·60 ± 0·11*, $n = 439$
Serum tryptophan, μmol/l	84·8 ± 13·7	93·3 ± 14·2*
Serum kynurenine, μmol/l	2·25 ± 0·56	2·56 ± 0·59*
Kyn/trp, μmol/mmol	27·0 ± 7·2	27·9 ± 7·3
Current or ex-smokers (%)	49·1	62·2*

trp: Tryptophan; kyn: kynurenine; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; BMI: body mass index; CRP: C-reactive protein; IMT: common carotid artery intima-media thickness. Values are mean ± s.d. unless indicated otherwise. *$P < 0·0001$, **$P = 0·004$, Student's *t*-test or χ^2 test.

concentration of CRP (logCRP) and kyn/trp as independent variables (Table 4). Kyn/trp was not an independent predictor for IMT in this multivariate model; the variables which remained associated significantly with IMT in female subjects were age and BMI.

Discussion

There is previous evidence from a small series ($n = 35$) of subjects with angiographically verified coronary heart disease that IDO activity is increased in patients with clinically evident coronary heart disease compared with healthy controls [13]. Increased carotid artery IMT measured by ultrasound is a surrogate marker for early atherosclerosis which predicts cardiovascular events in population groups [14]. We aimed here to test the hypothesis that IDO is involved in the immune regulation of early atherosclerosis in a large population-based sample of healthy young adults for whom both IMT measurements and detailed data on various cardiovascular risk factors were available.

Table 2. Correlations of risk factors for atherosclerosis with kyn/trp (μmol/mmol) in 544 young (24–39 years) female adults.

Variable	Correlation (r) with kyn/trp	P-value
Age	0·129	0·003
HDL	−0·090	0·037
LDL	0·111	0·010
logTriglyceride	−0·129	0·003
BMI, $n = 525$	0·219	< 0·0001
Waist circumference, $n = 531$	0·234	< 0·0001
Waist-to-hip ratio, $n = 529$	0·166	< 0·0001
Systolic blood pressure, $n = 541$	0·006	0·898
Diastolic blood pressure, $n = 541$	0·038	0·380
logCRP	0·085	0·048
IMT, $n = 541$	0·131	0·002

kyn: Kynurenine; trp: tryptophan; HD: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; logTriglyceride: logarithmically transformed concentration of serum triglyceride; BMI: body mass index; logCRP: logarithmically transformed concentration of C-reactive protein; IMT: common carotid artery intima-media thickness.

Table 3. Correlations of risk factors for atherosclerosis with kyn/trp (μmol/mmol) in 442 young (24–39 years) male adults.

Variable	Correlation (r) with kyn/trp	P-value
Age	0·066	0·163
HDL	−0·155	0·001
LDL	−0·013	0·791
logTriglyceride	0·017	0·718
BMI, $n = 433$	0·094	0·050
Waist circumference, $n = 441$	0·110	0·020
Waist-to-hip ratio, $n = 441$	0·103	0·030
Systolic blood pressure, $n = 438$	−0·086	0·072
Diastolic blood pressure, $n = 438$	−0·064	0·178
logCRP	0·234	< 0·0001
IMT, $n = 439$	0·040	0·402

kyn: Kynurenine; trp: tryptophan; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; logTriglyceride: logarithmically transformed concentration of serum triglyceride; BMI: body mass index; logCRP: logarithmically transformed concentration of C-reactive protein; IMT: common carotid artery intima-media thickness.

Table 4. Univariate correlations between risk factors for atherosclerosis and multivariate stepwise linear regression model of the relationships between the risk factors for atherosclerosis and IMT (mm) in 541 young (24–39 years) female adults.

| | Univariate analysis | | Multivariate analysis | |
| | Correlation (r) for IMT | | B $\pm$ s.e. | |
Risk variable	($n = 541$)	P-value	($n = 521$)	P-value
Age	0·278	< 0·001	0·004 $\pm$ 0·001	< 0·001
Kyn/trp	0·131	0·002		
HDL	−0·035	0·422		
LDL	0·080	0·063		
logTriglyceride	−0·062	0·148		
BMI, $n = 522$	0·147	0·001	0·002 $\pm$ 0·001	0·003
Systolic blood pressure	0·101	0·019		
Diastolic blood pressure	0·072	0·095		
logCRP	0·013	0·764		

IMT: common carotid artery intima-media thickness; kyn: kynurenine; trp: tryptophan; HD: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; logTriglycerid: logarithmically transformed concentration of triglyceride; BMI: body mass index; logCRP: logarithmically transformed concentration of C-reactive protein. Variables in the stepwise linear regression model: age, kyn/trp, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, logarithmically transformed concentration of triglyceride, body mass index, systolic blood pressure, diastolic blood pressure, logarithmically transformed concentration of C-reactive protein, smoking habits (current smoker or ex-smoker/never smoker).

Our main finding was that IDO activity correlated with IMT and several other risk factors for atherosclerosis in female subjects in univariate analysis, suggesting that IDO is involved in the regulation of the inflammatory response of early atherosclerosis. However, IDO activity did not have an independent effect on IMT in females when analysed in a multivariate model simultaneously with traditional risk factors for atherosclerosis and with CRP.

In addition to a significant correlation with IMT in females in univariate analysis, IDO activity correlated significantly with several risk factors for atherosclerosis. Similarly, in clinical epidemiological studies the marker of inflammation, CRP, has also been found to correlate with several conventional cardiovascular risk factors in women, including age, BMI, blood pressure and cigarette smoking [15] and to predict the occurrence of cardiovascular events, also among women [16]. However, there was only a weak correlation between IDO and CRP in females in the present study, implying that these markers of inflammation act differentially in the process of early atherosclerosis in females; neither IDO nor CRP correlated independently with IMT in a multivariate model in our cohort of young female adults. However, on the basis of the univariate analysis the IDO enzyme was a more potent predictor of carotid IMT in female subjects than CRP. In contrast, a clear correlation emerged between IDO and CRP in men, implying that these two separate markers of inflammation also act in a different way in females and males.

In addition to IDO, the degradation of tryptophan to kynurenine is also regulated by the hepatic enzyme tryptophan 2,3-dioxygenase (TDO) [9]. The possible role of TDO in the enhanced tryptophan degradation in the preclinical stages of atherosclerosis cannot be excluded, although IDO seems a more probable activator, as TDO regulates basal serum tryptophan concentrations and IDO is up-regulated in response to inflammatory conditions, which early atherosclerosis represents [9].

The positive correlation between IDO activity and age was to be expected, as IDO activity is known on the basis of previous studies to increase with age [17,18]. There was also a significant correlation between IDO and BMI, waist circumference and waist-to-hip ratio in both sexes. In parallel with this finding, in another recent study tryptophan depletion and thereby increased IDO activity was observed in morbidly obese patients, persisting even after weight reduction and leading to chronic immune activation [19].

In general, we do not know whether increased IDO activity is deleterious or advantageous in the process of development of early atherosclerosis. The first alternative is that increased IDO activity is purely a consequence of inflammation, i.e. merely a non-specific reactive marker of inflammation. However, if IDO activity is an attempt of the body to down-regulate the immune response induced by, e.g. ox-LDL or some other antigen, IDO would have a protective role against atherosclerosis. A specific type of T cells, i.e. regulatory T cells (T_{reg}), has been suggested recently to counteract disease initiation and progression in atherosclerosis [20]. IDO-expressing dendritic cells (DC) have in turn been postulated to generate T_{reg} [9]. If increased IDO activity induced T_{reg} cell activity in early atherosclerosis, increased IDO activity would constitute a favourable response.

However, as IFN-γ is one of the main inducers of IDO and as there is substantial evidence speaking for its importance in atherosclerosis (reviewed in [2]) it is also possible that IDO plays a pro-atherogenic role. At present, the nature of the role of IFN–γ/IDO interaction in atherosclerosis can only be speculated, as IDO expression can also be induced by an IFN-γ independent mechanism [21], and IFN-γ itself has both pro- and anti-atherogenic properties (reviewed in [22]).

In support of the alternative that IDO would protect from atherosclerosis, IFN-γ, a strong inducer of IDO, has been shown to inhibit oxidation of LDL-C in human mononuclear cells [23]. Here IDO activity correlated significantly with LDL-C in females, and it is possible that increased IDO activity is directed to down-regulate the immune response. Because the correlation between IDO activity and LDL-C was observed only in females, it would seem that this mechanism does not operate in the regulation of the inflammatory reaction of early atherosclerosis in males, but only in females. The inverse correlation between IDO activity and the anti-atherogenic HDL-C is in line with the finding of a direct correlation between LDL-C and IDO. However, the inverse correlation of IDO with triglyceride remains without explanation, particularly as there was a positive correlation between IDO and different indices of weight.

The fact that IDO was not a predictor of atherosclerosis in male subjects, as judged by the lack of correlation between IDO and IMT, suggests that hormonal factors might influence the effects of IDO. Oestrogen has indeed been found to up-regulate one type of APC, i.e. DC, to express IDO, which can limit T cell responses [24]. Moreover, IDO has been found to be elevated in autoimmune diseases with a female preponderance, for example systemic lupus erythematosus [25] and Sjögren's syndrome [26]. On the other hand, either early atherosclerosis or precociously increased IMT has been found in both these autoimmune diseases [27,28].

To conclude, our results suggest that the IDO enzyme is involved in the immune regulation of early atherosclerosis in young female adults. On the basis of our results we cannot definitely exclude the possibility that IDO is just another non-specific marker of inflammation, but we presume that IDO functions to down-regulate the inflammatory response, i.e. that its action is targeted to limit the inflammatory response. However, it is as such also a novel marker of immune activation in early atherosclerosis in females.

Acknowledgements

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