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Elimination of non-reactive and weakly reactive human α_1-acid glycoprotein after induction of the acute phase response in rats

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Abstract—The disposition of concanavalin A (Con A)-non-reactive and Con A-reactive human α_1-acid glycoprotein (AAG) was studied in normal male rats and in acute phase response-activated male rats. Activation of the acute phase response was made using a subcutaneous administration of ethynyloestradiol in sesame oil. This technique increased the endogenous AAG concentration 7-fold. In control rats the two forms of human AAG showed identical kinetics with an average clearance of 5·4 mL h^{-1} kg^{-1}, terminal half-life of 13·5 h and a volume of distribution (steady state) of 91 mL kg^{-1}. In the acute phase response-activated animals, both the clearance and volume of distribution were larger: the average clearance of the Con A-non-reactive AAG was 10·2 mL h^{-1} kg^{-1}, the volume of distribution (steady state) 152 mL kg^{-1} and the terminal half-life 11·7 h. The Con A-reactive AAG had a clearance of 14·7 mL h^{-1} kg^{-1}, a volume of distribution (steady state) of 262 mL kg^{-1} and a half-life of 15·8 h. The results indicate that not only does activation of the acute phase response alter the kinetics of AAG but that the change is different for the different types of AAG.

The plasma concentration of α_1-acid glycoprotein (AAG) is significantly increased in a number of disease states and physiological conditions (Routledge 1989) that activate the acute phase response (Kushner 1982). AAG is one of the slow reacting acute phase components reaching maximum plasma concentration between 1 and 5 days (Ashton et al 1970; Schmid 1975; Urban et al 1979; Kushner 1982) after activation, depending on the species. The increase is primarily dependent upon a significant increase in the mRNA of AAG (Ricca et al 1982) resulting in enhanced AAG synthesis. However, not only is the formation greater, the elimination is also increased during the acute phase response (Hellerstein & Munro 1987). However, the synthesis is increased more than elimination causing a net elevation of AAG in plasma.

AAG is heavily glycosylated with approximately 45% of the molecule consisting of carbohydrates (Schmid 1975). The carbohydrate component of human AAG consists of five oligosaccharide side chains having a variable degree of branching. The majority of the side-chains consist of tri- and tetra-antennary structures. If two or more side chains are in a bi-antennary form the protein interacts strongly with concanavalin A (Con A). With only one biantennary side chain it interacts only weakly with Con A, and with none, a Con A non-reacting protein is found (Bierhuizen et al 1988). In normal subjects the non-reacting AAG (AAG-A) contributes approximately 43% of the total AAG (Serbource-Goguel Seta et al 1986; Mackiewicz et al 1987, 1989; Pawlowski et al 1989). In many disease states the fraction of AAG-A may change significantly. For example, AAG-A has been reported to be 28–36% of total AAG in infections (Hansen et al 1989; Pawlowski et al 1989), 38% in renal failure and certain cancers (Hansen et al 1989), while it may be as high as 85% in pregnancy (Hansen et al 1989), 55–59% in severe rheumatoid arthritis (Mackiewicz et al 1987; Hansen et al 1989; Pawlowski et al 1989) but relatively normal in certain other cancers and pulmonary inflammation (Hansen et al 1989). The physiological consequences of these changes in the oligosaccharide side chains are not known but it has been reported that AAG in-vitro has a significantly higher degree of immune suppressing ability than does the Con A-reactive AAG (Durand 1989; Pos et al 1990).

The regulation of the branching is thought to be post-transcriptional although the mechanism is not clear. Changes in the glycosyl transferase concentrations, their co-factors, secretion of the completed AAGs and degradation of the various AAGs are all potential factors in the altered ratio of different AAGs. The secretion of the Con A-reactive and Con A-non-reactive AAG appears to be controlled by different mechanisms (Drechou et al 1989) with a decrease in the secretion half-life of the non-reactive AAG in rats with inflammation (Drechou et al 1989). However, the decrease is relatively small and is not expected to affect the extracellular concentration. Moreover, such a change would tend to increase the proportion of non-reactive AAG in the circulation, whereas most inflammatory conditions are associated with a decreased ratio.

This study was undertaken to determine whether activation of the acute phase response is associated with differences between the elimination of AAG-A and the reactive forms, and hence whether elimination is an important factor in changing the ratio of non-reactive to reactive AAG.

Materials and methods

Animals. Twenty-one male Sprague-Dawley rats, 275–305 g, were divided into four groups (two control, two experimental). The experimental groups were given 1 mg kg^{-1} ethynyloestradiol dissolved in 200 μL sesame oil, subcutaneously 36 h before the study to activate the acute phase response. One of the control groups (n = 5) and one of the experimental groups (n = 6) received approximately 20 mg kg^{-1} Con A-non-reactive human AAG (AAG-A). The two other groups (n = 5 in each) received approximately 20 mg kg^{-1} Con A-weakly reactive/reactive human AAG (AAG-B).

Experimental conditions. Rats were anaesthetized with a combination of 50 mg kg^{-1} ketamine and 5 mg kg^{-1} acepromazine (i.m.). Approximately 20 mg kg^{-1} AAG in 0·2 mL double-distilled water was administered i.v. via the penile vein and the animals allowed to recover. Blood samples (80 μL) were obtained in micro-capillary tubes via the tail vein just before and at 1, 2, 3, 8, 12, 23, 30 and 36 h after AAG administration. The animals had access to food and water throughout the experiment. The collected blood was centrifuged, the plasma separated and assayed by rocket (Axelsen & Bock 1983) and crossed immunoelectrophoresis (Bøg-Hansen 1983) using human AAG antibodies from rabbit (IgG fraction) (Sigma, St Louis, MO) and rat AAG antibodies from rabbit (whole serum) (BapCo, Berkely, CA).

AAG separation using Con A sepharose. Human AAG-A and AAG-B were separated from human AAG (Sigma) using the method of Bierhuizen et al (1988): a Con A sepharose column (8 cm × 1 cm i.d.) was equilibrated with 50 mL elution buffer A (Trizma base 0·05 M, NaCl 1 M, $MgCl_2$ 1 mM, $CaCl_2$ 1 mM, $MnCl_2$ 1 mM).

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Twenty five mg of human AAG (Sigma) in 0·5 mL elution buffer A was placed on the top of the column and eluted with elution buffer A. Sixteen 1 mL fractions were collected and the elution was continued with elution buffer B (elution buffer A containing 75 mM methyl-α,d-mannopyranoside) and an additional sixteen 1 mL fractions were collected. The fractions with significant absorption at 280 nm were pooled, desalted on a PD 10 Sephadex column (Pharmacia, Uppsala, Sweden) and lyophilized. Crossed immunoelectrophoresis indicated that AAG-A contained only Con A-non-reactive AAG, whilst AAG-B contained approximately 80% weakly-Con A-reactive and 20% highly Con A-reactive AAG.

Results

Induction of the acute phase response resulted in an approximately 7-fold (34·5 ± 6·2 vs 5·1 ± 2·5 μM) increase in the rat AAG plasma concentration measured 24 h after ethynyloestradiol administration. The ratio of Con A-non-reactive to reactive forms of AAG (AAG-A/AAG-B) decreased from 0·12 ± 0·06 to 0·07 ± 0·05%, but did not reach statistical significance (t = 1·83). The animals showed no outward sign of discomfort after administration of the ethynyloestradiol solution and appeared normal throughout the experiment.

The plasma concentration of human AAG-A and AAG-B both in the control and treated animals was biphasic. The log-averaged concentrations in the four groups are given in Fig. 1 and the corresponding pharmacokinetic parameters in Table 1.

The most significant differences seen among the groups are a higher clearance and a larger apparent volume of distribution in the acute phase-activated animals in comparison with normal rats (Table 1, $P < 0·001$ and $P < 0·01$, respectively). Both the initial and steady state apparent volumes of distribution were larger in the acute phase activated animals (Table 1, $P < 0·01$ and $P < 0·002$, respectively). In addition, the ratio of steady state volume of distribution to initial distribution volume was 1·65 ± 0·21 in the control animals compared with 1·94 ± 0·32 in the induced animals ($P < 0·05$). Because volume of distribution and clearance were both greater in the induced rats, the half-life and the mean residence times were not statistically significantly different between the groups ($P > 0·50$).

In control animals the values for all pharmacokinetic parameters were almost identical between AAG-A and AAG-B (Table 1). In the acute phase-activated animals AAG-B had a marginally higher clearance value ($P = 0·05$), a larger initial distribution volume ($P < 0·02$) and a marginally larger steady state volume of distribution ($P < 0·05$) than AAG-A.

Discussion

The pharmacokinetic parameters of AAG in the control animals are similar to those obtained by Keyler et al (1987) in the rat, for human AAG. In our study the average clearance was 5·4 mL h^{-1} kg^{-1} and in the study by Keyler et al 6·5 mL h^{-1} kg^{-1}. (In the original report by Keyler et al the value is quoted as 0·065 L h^{-1} kg^{-1}, but the fitted parameters indicate that the units should be dL min^{-1} kg^{-1} and not L h^{-1} kg^{-1}.) Keyler et al reported a terminal half-life of 19·3 h and volume of distribution (steady state) of 150 mL kg^{-1} vs 13·5 h and 91 mL kg^{-1}, respectively, in this study. The doses in the study of Keyler et al were 100 times larger than in this study and the similarity between the results would, therefore, tend to indicate that the disposition of AAG is not dose-dependent.

Our studies indicate that the clearance of AAG is significantly increased after activation of the acute phase response. The reason for the increased elimination is not clear. Whether this relates to an increased hydrolysis of the sugar side chain, e.g. by neuraminidase, and faster removal of the asialo AAG is an intriguing possibility but needs further investigation. Increased neuraminidase activity has been associated with situations where the acute phase response has been activated, such as infarction (Hanson et al 1987) and intestinal cancers (Corfield et al 1983); in other situations where the acute phase response should be activated, in hepatomas and regenerations of liver damage, neuraminidase activity has been found to be decreased (Sagawa et al 1988).

The increase in apparent volume of distribution in the acute phase activated animals was unexpected. We may postulate two

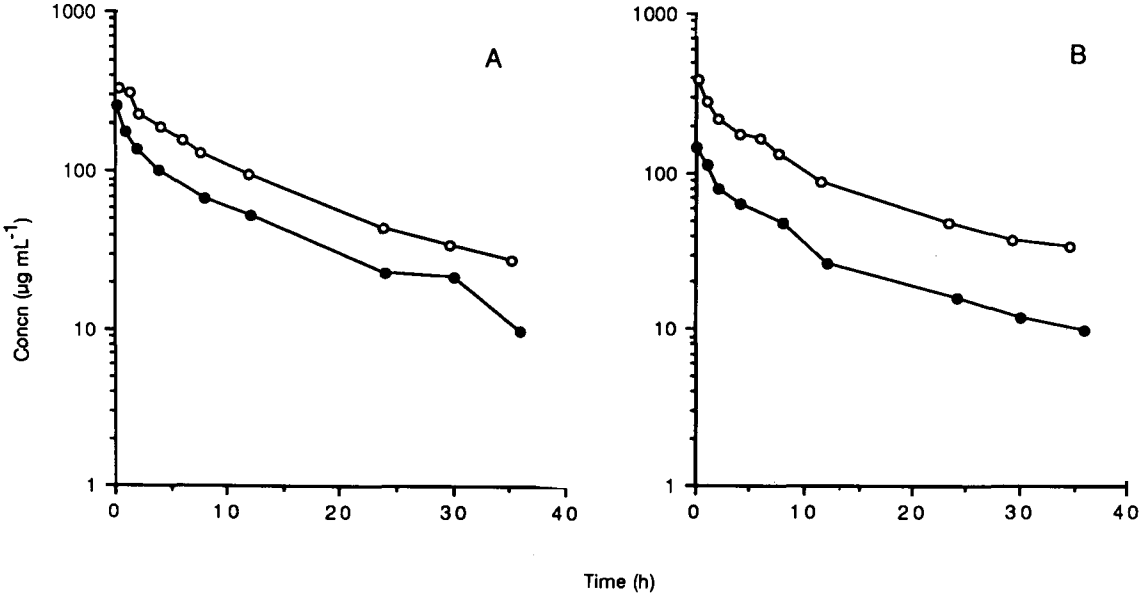

FIG. 1. Plasma concentration of different human AAGs in control rats (○) and in acute phase-induced rats (●). A. Administration of non-reactive human AAG. B. Administration of reactive human AAG.

Table 1. Pharmacokinetic parameters of human AAG-A and AAG-B in normal rats and in acute phase-activated rats.

Pharmacokinetic parameters	Control rats		Acute phase-activated rats	
	AAG-A	AAG-B	AAG-A	AAG-B
Clearance (mL h^{-1} kg^{-1})	5·4 ± 1·4	5·4 ± 1·1	10·2 ± 4·1	14·7 ± 1·8
Vd_c (mL kg^{-1})	57 ± 12	53 ± 14	76 ± 29	141 ± 41
Vd_{ss} (mL kg^{-1})	90 ± 30	91 ± 16	152 ± 69	262 ± 81
Half-life (h)	13·8 ± 4·3	13·3 ± 1·3	11·7 ± 3·0	15·8 ± 5·2
MRT (h)	16·9 ± 4·3	17·0 ± 1·8	15·0 ± 3·0	17·7 ± 3·6
n	5	5	6	5

Vd_c = the initial apparent distribution volume; Vd_{ss} = the apparent distribution volume at steady state; MRT = mean residence time. Values are mean ± s.d.

explanations: an increased diffusion of AAG from the vascular system with a higher fraction in the extravascular space; or an increased cellular association of AAG again resulting in an increased extracellular distribution. It is known that inflammation causes an increased capillary leakage (Joris et al 1990) which will elevate the extracellular concentration of vascular compounds. However, if leakage were the sole explanation, then the leakage of AAG-B would need to be significantly greater than for AAG-A. Although the mol. wt of AAG-B is less than that of AAG-A, it is only marginally so and conformational effects are expected to have a greater influence. Vertebrates are known to have a number of lectin-like compounds that are able to bind glycosylated proteins. Some of these are proteins involved with the acute phase response (Ezekowitz et al 1988; Lasky 1991). Whether these lectin-like compounds have different affinities for proteins with different degrees of branching is speculative but could explain the difference in the apparent volume of distribution seen between AAG-A and AAG-B during activation of the acute response.

Our results suggest that the large change in the ratio of the Con A-reactive to non-reactive AAG seen after activation of the acute phase response is not likely to be due to a change in the elimination of these two groups of AAG. The slightly higher clearance of AAG-B in comparison with AAG-A would suggest that an increased ratio of AAG-A to AAG-B is expected if the formation is otherwise constant. The ratio of AAG-A to AAG-B is generally decreased after induction of the acute phase response; this was observed in the rat AAG profiles. Thus we suggest that AAG-B formation is increased to a greater extent than AAG-A formation in the acute phase response. These suggestions should, however, be viewed with some caution as deductions are from studies of human AAG in the rat.

This study does not give information of the kinetics of the highly Con A-reactive AAG, which comprised approximately 20% of the administered AAG-B. Eight hours after administration of AAG-B the highly Con A-reactive AAG still represented approximately 20% of the human AAG in plasma both in the control and ethynyloestradiol-treated animals. Beyond this time it was not possible to quantitate the highly Con A-reactive AAG with any degree of accuracy after separation from the intermediate reactive-AAG using crossed immuno-electrophoresis. Whether the highly reactive and intermediate reactive forms of AAG have different dispositions is therefore not ascertainable from this study.

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Ageing influences haloperidol-induced changes in the permeability of the blood-brain barrier in the rat

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Abstract—The effect of the dopaminergic antagonist haloperidol on the permeability of the blood-brain barier (BBB) to [^{14}C]α-aminoisobutyric acid was studied in 10–12- and 28–30-week old rats. Following the intraperitoneal injection of haloperidol (1 mg kg^{-1}), an increase in the permeability of the BBB, with respect to younger animals, was observed within the occipital cortex, striatum, hippocampus and hypothalamus in the older rats. No correlation was found between haloperidol-induced changes and age-related differences in the permeability of the BBB. Such age-associated increase in the vulnerability of the BBB when challenged with haloperidol might be related to a deterioration of the dopaminergic control of cerebrovascular permeability.

The blood-brain barrier (BBB) acts as a selectively permeable membrane able to maintain normal ionic differences between the blood and brain extracellular fluid (Rapoport 1976; Pardridge 1987, 1988); clearly the structural and functional integrity of the BBB is essential to normal neurological function. Apart from several known mechanisms by which the BBB breaks down, other circumstances can alter the non-specific permeability of the BBB, in the absence of ultrastructurally visible tearing of endothelial membranes and of gross changes or degeneration processes of the vascular patterns of the brain (Rapoport et al 1972; Petito et al 1982; Ellison et al 1986). In particular, we reported ageing-related modifications in the permeability of the BBB, probably reflecting alterations in brain neurochemical systems (Saija et al 1990a). In addition, there is wide evidence that centrally-acting drugs, such as amphetamine (Sankar et al 1983), arecoline (Saija et al 1990b), pentobarbitone and ketamine (Saija et al 1989), can induce modifications in the normal functioning of the BBB through different and perhaps concomitant mechanisms (direct action at the level of receptors present in the cerebral capillary endothelium and subserving vasoregulatory responses, changes in the neurogenic component controlling BBB permeability, alterations in the release of vasoactive substances). Furthermore, few data are reported concerning ageing-related changes in the effects of centrally acting drugs on the permeability of the BBB. The present study was undertaken to compare the changes in the functional activity of the BBB induced by the dopaminergic antagonist haloperidol in young and aged rats. Determination of the permeability of the BBB involved the use of a small molecular weight radiolabelled amino acid, [^{14}C]α-aminoisobutyric acid ([^{14}C]AIB); this tracer is only minimally transported across normal cerebral microvessels and is actively transported into the cells, so that the isotope is trapped in viable parenchymal cells (Blasberg et al 1980, 1983a, b; Gross et al 1982; Picozzi et al 1985; Saija et al 1990b), allowing the precise localization and quantitative expression of BBB changes.

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Materials and methods

Animal preparation. Male Wistar rats, 10–12-weeks old, 220–240 g, and 28–30-weeks old, 450–500 g, were kept under standardized conditions, with free access to food and water, and a 12 h light/dark period (light on: 0600 h).

On the day of the experiment, the animals were anaesthetized with pentobarbitone sodium (54 mg kg^{-1}, i.p.) and spontaneously breathed room air. Short PE20 polyethylene catheters filled with 100 int. units mL^{-1} heparin in 0·9% NaCl (saline) were inserted into the left femoral vein and artery for blood sampling and administration of tracers. Before isotope injection and periodically during the experiment, arterial blood samples were withdrawn for measurements of arterial blood gases and pH. Body temperature was maintained at 37°C by external heating. All experiments were carried out in the morning, between 0900 and 1100 h.

BBB permeability. Details of techniques for determination of the permeability of the BBB have been presented previously (Saija et al 1989, 1990a, b). A bolus of 15–25 μCi [^{14}C]AIB was injected intravenously. Blood was collected periodically from the femoral artery until the rat was killed by decapitation 30 min after the injection of the tracer. A large blood volume was withdrawn at the end to measure the whole-blood isotope concentration; 30 min was chosen to minimize the effect of intravascular tracer on brain ^{14}C activity measured at the end of the experiment, and, also, the eventual brain-to-blood reflux of tracer. The brain was rapidly removed and dissected into 8