Br. J. clin. Pharmac. (1989), **27**, 257–261

Lack of effect of treatment with human recombinant-tumour necrosis factor (HrTNF) on the binding of quinidine to α_1-acid glycoprotein (AGP)

JANICE A. BARNETT[1], D. E. BRENNER[2,3], P. J. CREAVEN[2] & D. LALKA[1]
[1]Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo; [2]Department of Clinical Pharmacology and Therapeutics, Roswell Park Memorial Institute, and the [3]Department of Medicine, State University of New York at Buffalo, Buffalo, New York, USA

Tumour necrosis factor (TNF) is known to be a key mediator in the acute phase response and its administration has been shown to cause a five fold increase in serum α_1-acid glycoprotein (AGP) concentration in the rat. Since, in man, plasma AGP level determines the protein binding of many important drugs (e.g. narcotic analgesics, phenothiazines, antiarrhythmics, calcium channel blockers) likely to be given to patients who will be treated with TNF, it is important to determine if TNF treatment of humans causes a similar increase in AGP concentration and drug binding. Therefore, the plasma protein binding of quinidine and the serum level of AGP were studied over a 4 day period in each of five cancer patients who were treated with human recombinant-tumour necrosis factor (HrTNF) using a dosage schedule of 6–8 $\times$ 10^{+5} units/m^2 daily for 5 days. It was observed that the quinidine binding ratio (the quotient of bound and free concentration in plasma) was highly correlated with the plasma concentration of AGP (r = 0.818) and that the mean pretreatment AGP concentration in the patients was about three times that found in normal subjects. However, no effects of the TNF treatment regime used in the present study could be demonstrated on either plasma AGP concentration or quinidine free fraction. These observations allow the *tentative* conclusion that HrTNF does not cause a significant increase in serum AGP level in cancer patients whose baseline AGP concentration is high. However, further study of the relationship between TNF treatment and serum AGP level is needed.

Keywords tumour necrosis factor α_1-acid glycoprotein plasma protein binding quinidine binding acute phase response

Introduction

Biological response modifiers such as tumour necrosis factor (TNF) are currently of interest as potential antineoplastic agents (Creaven *et al.*, 1987; Old, 1987). TNF elicits many effects including macrophage activation, direct cytotoxicity to some types of tumour cells, protection of mice from certain malarial pathogens as well as causing some of the symptoms associated with the injection of foreign proteins (Creaven *et al.*, 1987; Dinarello & Mier, 1987; Old, 1987).

Correspondence: Dr David Lalka, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260, USA

Furthermore, as a part of its well documented role in the acute phase response, TNF causes profound increases (2–20 fold) in the serum concentration of many proteins including haptoglobin, C-reactive protein, fibrinogen and α_2-macroglobulin (Perlmutter *et al.*, 1986; Gresser *et al.*, 1987).

The concentration of α_1-acid glycoprotein (AGP) is the principal determinant of the protein binding of many basic lipophilic drugs (e.g. propranolol, quinidine, most phenothiazine antiemetics, many narcotic analgesics) in human serum (Piafsky *et al.*, 1978; Svensson *et al.*, 1986). Reports concerning the effect of TNF on the serum concentration of this particular acute phase protein are conflicting. It has been reported that the administration of recombinant murine TNF (20 μg; i.p.) to rats causes a five fold increase in serum AGP concentration (Gresser *et al.*, 1987) but studies in cultured rat hepatocytes suggest only moderate increases in the production of this protein (Koj *et al.*, 1987). Similarly, cultured human hepatoma cells either did not increase their rate of synthesis of AGP (Darlington *et al.*, 1986) or exhibited a small increase (Perlmutter *et al.*, 1986) when exposed to TNF. Clearly the results of such experiments could be influenced by many factors including the dose of TNF and the presence of other biological response modifiers (Old, 1987; Darlington *et al.*, 1986). Based on the positive response of rats to the *in vivo* administration of TNF and the substantial evidence suggesting that TNF has a major role in the acute phase response in several species, it seemed appropriate to examine the effect of human recombinant-tumour necrosis factor (HrTNF) treatment on plasma AGP concentration in patients who were receiving this compound in the course of a phase 1 clinical trial. Thus, if TNF administration caused plasma AGP concentration to increase markedly, it would be expected to decrease the free concentration of parenterally administered (but probably not orally administered) drugs which exhibit blood flow rate limited hepatic clearance (Wilkinson & Shand, 1975). Examples of such compounds which would be expected to be extensively used in a patient population likely to receive TNF include many phenothiazines and narcotic analgesics. Most available data suggest that such changes in free concentration would be expected to be associated with a decreased pharmacologic effect (Huang & Øie, 1982; De Rick *et al.*, 1987). For these reasons, we performed a study of the effect of treatment with HrTNF on the plasma concentration of AGP in humans and on the binding of a model lipophilic base (quinidine) in human plasma.

Methods

Five male patients, 40 to 65 years of age (median 56 years), received intravenous infusions (1 h) of HrTNF (Asahi Chemical Industry Co., Ltd, The Imperial Tower, Tokyo 100, Japan) at 24 h intervals for 5 days at doses of 6–8 $\times$ 10^{+5}U m^{-2}. Four of the five patients had renal cell carcinoma and the remaining subject had a carcinoma of the pancreas. All patients gave written informed consent before entry to the study and the project was approved by the Roswell Park Memorial Institute Clinical Investigation Committee (Institutional Review Board).

Heparinized blood samples were collected prior to administration of the first TNF dose and the subsequent samples were collected 24, 48, 72 and 96 h later (just before the 2nd, 3rd, 4th and 5th doses of TNF). Plasma was separated by centrifugation and stored at −20° C. A review of the charts of these patients confirmed that they received no drugs known to influence plasma AGP concentration in man. Furthermore, the doses of all drugs known to bind to AGP were sufficiently low such that any drug-drug competitive interaction was unlikely (Goolkasian *et al.*, 1983). Specifically, two patients received pethidine and one each received lignocaine, prochlorperazine and chlorpromazine. The details of the displacement of lignocaine by chlorpromazine, pethidine, quinidine and ten other lipophilic basic drugs as well as a detailed assessment of the lignocaine-quinidine interaction have been reported previously from this laboratory (McNamara *et al.*, 1981; Goolkasian *et al.*, 1983). It is clear from these data and considerations derived from the law of mass action coupled with a knowledge of the concentrations of lignocaine, pethidine, chlorpromazine and prochlorperazine which could be achieved in patients (without causing severe toxicity), that no significant drug-drug protein binding displacement interaction should be anticipated.

For the control of fever, ketoprofen (50 mg p.o.) was given to all subjects on the evening preceding the first administration of TNF and at 08.00 h, 13.00 h, and 22.00 h each day for 5 days.

AGP concentration in plasma was measured in each sample using a commercially available radial immunodiffusion assay kit (Behring Diagnosis, La Jolla, CA). The free fraction of quinidine in plasma was measured by ultrafiltration (Amicon-Centrifree® system) of 1.0 ml of plasma to which quinidine (2 μg/ml) had been added *in vitro*. The pH of each sample was adjusted to 7.40 $\pm$ 0.05 with 0.5 N H_3PO_4 since it had previously been reported that the free fraction of at least some lipophilic bases is highly

dependent on the hydrogen ion activity in serum (McNamara *et al.*, 1981). Samples were subsequently centrifuged for 15 min in a temperature controlled unit (25° C) at 1250 *g*. The concentration of quinidine was measured in the ultrafiltrates using a modification (Edwards *et al.*, 1987) of a previously reported h.p.l.c. method (Drayer *et al.*, 1977). The only other changes made were related to sample preparation. Briefly, 20 μl of an aqueous internal standard solution (quinine 2 μg ml^{-1}) was added to 100 μl of ultrafiltrate and gently vortexed. Sixty μl of the mixture was injected and fluorescence intensity was measured using an excitation wavelength of 340 nm. The quinidine free fraction was calculated as the ratio of unbound to total quinidine concentration in each sample. All data are presented as the population mean ± one standard deviation unless otherwise indicated.

Results

As has been reported previously for a population of cancer patients (Ward *et al.*, 1975), the pretreatment plasma AGP concentration was much higher (2.23 ± 0.94 g l^{-1}) than that observed in a population of normal healthy subjects (~0.7 g l^{-1}). Consistent with this, the mean quinidine free fraction in our patient population (0.077 ± 0.017) was about half of that which we had observed in a sample of normal subjects (Edwards *et al.*, 1984). There was a strong positive correlation ($r = 0.818$) between the quinidine binding ratio (bound concentration divided by free concentration) and plasma AGP concentration (data not shown). Of greatest importance, the data in Figure 1 suggest that treatment with HrTNF did not cause an increase in plasma AGP concentration or change the free fraction of quinidine.

Discussion

Therapeutic and toxic effects are often correlated more closely with free drug concentration in plasma than with the total plasma concentration of drug (Huang & Øie, 1982; De Rick *et al.*, 1987; Svensson *et al.*, 1986). Thus, determining the effect of systemic administration of biological response modifiers such as TNF on drug binding may be important because such treatment might alter the plasma concentration of many acute phase proteins and hence drug effect. The observation that the HrTNF preparation (at the doses employed) used in the present investigation did not significantly affect plasma AGP

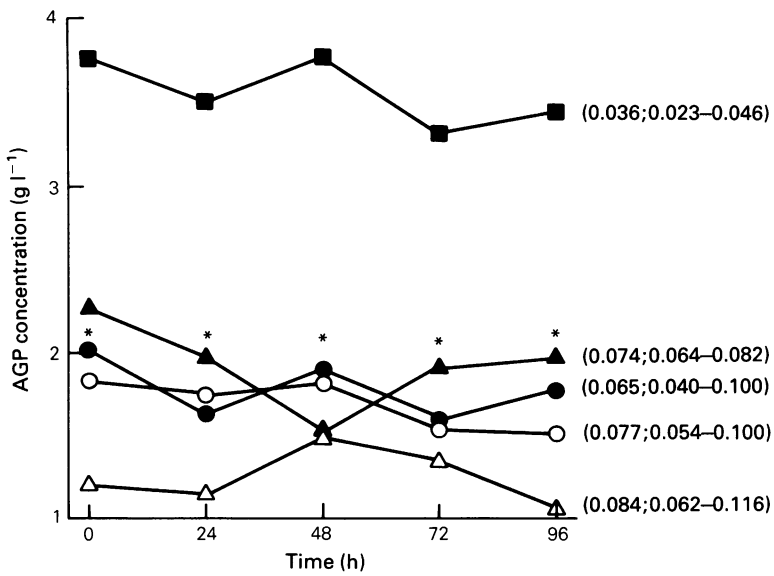

Figure 1 Plasma α_1-acid glycoprotein concentration during a 4 day treatment period with HrTNF (6–8 × 10^{+5} units m^{-2} daily × 5 days). Each symbol represents the results from a single patient and the numbers given in the parenthesis at the end of each curve are the mean quinidine free fraction observed in that individual followed by the range of free fractions which were observed over the study period. The asterisks are the mean AGP concentrations from all subjects at the various time points.

concentration or quinidine free fraction values may reflect the small number of subjects studied and the dosing schedule used. However, the results of previous investigations on trauma patients performed in this laboratory (Edwards *et al.*, 1982; Kirkwood *et al.*, 1986) suggest that the rate of increase in plasma AGP concentration is quite predictable (i.e. it increases ~0.25 $g\,l^{-1}day^{-1}$ for the first 5–7 days post trauma) and that normal subjects exhibit stable AGP concentrations over periods of time such as that employed in this study (Edwards *et al.*, 1982). Thus, given an adequate stimulus, humans exhibit profound increases in the plasma concentration of AGP.

The negative results observed in the present investigation suggest a number of possibilities in addition to those already discussed. First, in man, unlike the rat (Gresser *et al.*, 1987) TNF may not be responsible for the increase in AGP concentration which accompanies the acute phase response. It is also possible that patients with advanced neoplastic disease already exhibit a near maximal rate of AGP synthesis (our subjects had preteatment AGP levels which averaged about three times the concentration seen in normal subjects). Thus, a further stimulus to AGP production may not have a measurable effect. While we cannot rule out this possibility, a subject in the present study who exhibited a near normal plasma AGP concentration before the initiation of TNF treatment did not demonstrate a significant response to this treatment. Finally, it should be noted that even if the specific product of recombinant DNA technology which we investigated was devoid of this particular activity, increased serum AGP concentration could still be associated with native TNF since it is possible that minor differences in protein structure exist (Wang *et al.*, 1985). Several different biological response modifiers (TNF, interleukin-1, various interferons, etc.) may play a role in increasing serum AGP concentration in man. It is prudent, therefore, to perform a systematic assessment of the effect of each of these agents on plasma AGP concentration as they enter early clinical trials.

This work was supported in part by Grant GM-20852 from the Institute of General Medical Sciences, National Institutes of Health, by a Ciba-Geigy American Foundation for Pharmaceutical Education Fellowship (J.A.B.), and Asahi Chemical Industries, Ltd, Tokyo, Japan.

References

Creaven, P. J., Plager, J. E., Dupere, S., Huben, R. P., Takita, H., Mittelman, A. & Proefrock, A. (1987). Phase I clinical trial of recombinant human tumor necrosis factor. *Cancer Chemother. Pharmac.*, **20**., 137–144.

Darlington, G. J., Wilson, D. R. & Lachman, L. B. (1986). Monocyte-conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. *J. cell Biol.*, **103**, 787–793.

De Rick, A. F., Belpaire, F. M., Dello, C. & Bogaert, M. G. (1987). Influence of enhanced alpha-1-acid glycoprotein concentration on protein binding, pharmacokinetics and antiarrhythmic effect of lidocaine in the dog. *J. Pharmac. exp. Ther.*, **241**, 289–293.

Dinarello, C. A. & Mier, J. W. (1987). Current concepts: Lymphokines. *New Engl. J. Med.*, **317**, 940–945.

Drayer, D. E., Restivo, K. & Reidenberg, M. M. (1977). Specific determination of quinidine and (3S)-3-hydroxyquinidine in human serum by high-pressure liquid chromatography. *J. lab. clin. Med.*, **90**, 816-822.

Edwards, D. J., Axelson, J. E., Slaughter, R. L., Elvin, A. T. & Lalka, D. (1984). Factors affecting quinidine protein binding in man. *J. pharm. Sci.*, **73**, 1264–1267.

Edwards, D. J., Axelson, J. E., Visco, J. P., vanEvery, S., Slaughter, R. L. & Lalka, D. (1987). Lack of effect of smoking on the metabolism and pharmacokinetics of quinidine in patients. *Br. J. clin. Pharmac.*, **23**, 351-354.

Edwards, D. J., Lalka, D., Cerra, J. & Slaughter, R. L. (1982). Alpha$_1$-acid glycoprotein concentration and protein binding in trauma. *Clin. Pharmac. Ther.*, **31**, 62-67.

Goolkasian, D. L., Slaughter, R. L., Edwards, D. J. & Lalka, D. (1983). Displacement of lidocaine from serum α_1-acid glycoprotein binding sites by basic drugs. *Eur. J. clin. Pharmac.*, **25**, 413-417.

Gresser, I., Delers, F., Tran Quangs, N., Marion, S., Engler, R., Maury, C., Soria, C., Soria, J., Fiers, W. & Tavernier, J. (1987). Tumor necrosis factor induces acute phase proteins in rats. *J. Biol. Regul. Homeost. Agents*, **1**, 173-176.

Huang, J. D. & Øie, S. (1982). Effect of altered disopyramide binding on its pharmacologic response in rabbits. *J. Pharmac. exp. Ther.*, **223**, 469–471.

Kirkwood, C. F., Edwards, D. J., Lalka, D., Lasezkay, G. M., Hassett, J. M. & Slaughter, R. L. (1986). The pharmacokinetics of meperidine in acute trauma patients. *J. Trauma*, **26**, 1090–1093.

Koj, A., Kurdowska, A., Magielska-Zero, D., Rokita, H., Sipe, J. D., Dayer, J. M., Demczuk, S. & Gauldie, J. (1987). Limited effects of recombinant human and murine interleukin 1 and tumor necrosis factor on production of acute phase proteins by cultured rat hepatocytes. *Biochem. Int.*, **14**, 553–560.

McNamara, P. J., Slaughter, R. L., Pieper, J. A., Wyman, M. G. & Lalka, D. (1981). Factors influencing the serum protein binding of lidocaine in humans. *Anesth. Analg.*, **60**, 395–400.

Old, L. J. (1987). Tumor necrosis factor: Polypeptide mediator network. *Nature*, **326**, 330–331.

Perlmutter, D. H., Dinarello, C. A., Punsal, P. I. & Colten, H. R. (1986). Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. *J. clin. Invest.*, **78**, 1349–1354.

Piafsky, K. M., Borga, O., Odar-Cederlof, I., Johansson, C. & Sjoqvist, F. (1978). Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma α_1 acid glycoprotein. *New Engl. J. Med.*, **299**, 1435–1439.

Svensson, C. K., Woodruff, M. N., Baxter, J. G. & Lalka, D. (1986). Free drug level monitoring in clinical practice: Rationale and current status. *Clin. Pharmacokin.*, **11**, 450-469.

Wang, A. M., Creasey, A. A., Ladner, M. B., Lin, L. S., Strickler, J., vanArsdell, J. N., Yamamoto, R. & Mark, D. F. (1985). Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science*, **228**, 149-154.

Ward, A. W., Cooper, E. H., Turner, R., Anderson, J. A. & Nelville, A. M. (1975). Acute-phase reactant protein profiles: an aid to monitoring large bowel cancer by EA and serum enzymes. *Br. J. Cancer*, **35**, 170-178.

Wilkinson, G. R. & Shand, D. G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmac. Ther.*, **18**, 377-390.

(Received 18 May 1988, accepted 22 September 1988)