Pharmacological interference with tissue hypercatabolism in tumour-bearing rats

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Marked loss of body weight and profound waste of both skeletal muscle and white adipose tissue occur in rats into which the ascites hepatoma Yoshida AH-130 has been transplanted, associated with marked perturbations in the hormonal homoeostasis and the presence of circulating tumour necrosis factor and high plasma levels of prostaglandin E_2 [Tessitore, Costelli and Baccino (1993) Br. J. Cancer **67**, 15–23]. On the basis of previous findings, the present study examined whether the development of cachexia in this model system could be significantly affected by adrenalectomy or by pharmacological treatments that may interfere with proximal or distal mediators of tissue hypercatabolism. In no instance was tumour growth modified. Medroxyprogesterone acetate, an anabolic-hormone-like drug, was completely ineffective. In adrenalectomized animals, although changes such as the elevation of plasma triacylglycerols and corticosterone were corrected, the general course of cachexia was not modified. A partial prevention of muscle waste was observed with acetylsalicylic acid, a non-steroidal anti-inflammatory drug, or with leupeptin, a proteinase inhibitor. Insulin afforded the most significant preservation of muscle protein and adipose-tissue mass, which were maintained close to control values even 10 days after transplantation. The effects of insulin on gastrocnemius muscle and liver protein content were exerted by slowing down protein turnover, mainly enhancing synthesis. Consistently, the total free amino acid concentration in the gastrocnemius of insulin-treated rats 10 days after tumour transplantation was close to that of controls. Although treatment with insulin decreased plasma corticosterone to normal values, it did not modify the circulating level of tumour necrosis factor. On the whole these data show that it seems possible to prevent, at least in part, the tissue waste that characterizes cancer cachexia by purely pharmacological means.

INTRODUCTION

Cancer cachexia is a complex syndrome wherein severe, progressive, loss of body weight is mainly accounted for by wasting of host body compartments such as skeletal muscle and adipose tissue. Weight loss and tissue wasting are observed in up to two-thirds of patients dying from cancer (Kern and Norton, 1988), and weight loss implies poorer prognosis and shorter survival time for cancer patients (De Wys, 1985). Moreover, the management of cachexia raises serious, largely unresolved, problems.

In dealing with clinical or experimental cancer cachexia, most approaches imply the assumption, even if not openly stated, that the underlying disturbances can be largely equated to a situation of malnutrition accounted for by factors such as hypophagia, malabsorption or metabolic competition by tumour cells. Total parenteral nutrition has thus been extensively evaluated as a supportive therapy to reverse cachexia (Brennan, 1981; Rumley and Copeland, 1985), yet proved disappointing in tumour-bearing animals (Cameron, 1981; Popp et al., 1981, 1984) as well as in cancer patients (Chlebowski, 1985; Bozzetti et al., 1987; Shaw and Wolfe, 1988; Hyltander et al., 1991). Moreover, although the inability to utilize food conveniently is accompanied by a devastating breakdown of the lean body mass (Lundholm et al., 1976), a potential pitfall of total parenteral nutrition is that the tumour itself may benefit by the extra nutrients supplied (Cameron, 1981; Baron et al., 1986).

As worked out in our laboratory, the rat ascites hepatoma Yoshida AH-130 has proved a very convenient model to study cancer cachexia. This develops early after tumour transplantation, in association with enhanced tissue protein catabolism (Tessitore et al., 1987, 1993a), adipose-tissue depletion (N. Carbó, P. Costelli, L. Tessitore, G. J. Bagby, F. J. Lopez-Soriano, F. M. Baccino and J. M. Argilés, unpublished work) and humoral perturbations all poised in a catabolic sense, such as decrease of plasma insulin (INS), elevation of counter-regulatory mediators [glucagon, corticosterone, catecholamines and prostaglandin E_2 (PGE_2)], and presence of circulating tumour necrosis factor (TNF), a putative catabolic cytokine (Tessitore et al., 1993b). Data from another laboratory are consistent with such a general pattern (Strelkov et al., 1989; Baracos et al., 1992). Neither the observed decrease in food intake (Tessitore et al., 1993a,b) nor the tumour–host competition (Tessitore et al., 1987, 1993a,b) could by themselves account for the metabolic derangement and tissue wasting in tumour bearers. Rather, these changes appeared largely determined by a complex network of humoral factors that all forced tissue metabolism, particularly protein turnover, into a catabolic setting.

The possibility of interfering with the onset of the main metabolic disturbances in AH-130 tumour-bearing rats has been investigated in the present study. In view of our previous observations, the rationale was to scrutinize some treatments selected for their ability to interfere with factors or processes putatively involved in establishing the protein hypercatabolic state. It is shown below that, although administration of medroxyprogesterone acetate (MPA) or adrenalectomy (ADX) had no appreciable beneficial consequences, treatments with INS, acetylsalicylic acid (ASA) or leupeptin (LPP) were all effective in preventing, at least partially, the loss of body weight and waste

Abbreviations used: ADX, adrenalectomy; ASA, acetylsalicylic acid; INS, insulin; LPP, leupeptin; MPA, medroxyprogesterone acetate; PGE_2, prostaglandin E_2; TNF, tumour necrosis factor.

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of skeletal-muscle protein in AH-130 tumour-bearing rats. Moreover, INS and ASA were also very effective in counteracting the adipose-tissue depletion. The present observations thus further support the view (Tessitore et al., 1987, 1993a,b) that cachexia in this model mostly results from a tissue hypercatabolic state developing in the course of the tumour–host interaction.

EXPERIMENTAL

The experiments were performed on male Wistar rats weighing about 200 g (Nossan, Milano, Italy), having unrestricted access to food and water, and maintained under a 12 h-light/12 h-dark cycle. The diet (Piccioni, Brescia, Italy) was constituted of 49% carbohydrates, 20% protein and 5% fat; the rest was non-digestible material. Rats receiving an intraperitoneal inoculum of 5×10^7 Yoshida ascites-hepatoma AH-130 cells showed an appreciable body weight loss as early as 48 h after transplantation, and died in about 15 days (cf. Tessitore et al., 1987). Both tumour-bearing and non-tumour-bearing rats were divided into two groups for each treatment (controls and treated). The daily food intake of tumour bearers was measured, and pair-fed controls were provided with the same amount of food.

INS and LPP were given since day 1 after transplantation by subcutaneous injections, the former daily (15 units/kg body wt.) and the latter 8-hourly (2.5 mg/kg body wt.) for a total of 4 (either drug) or 10 days (INS only). A priming dose of ASA (6 mg/kg body wt., subcutaneously) was followed by a daily maintenance dose (40 mg/kg body wt.) delivered for 4 or 7 days with an osmotic minipump (Alzet, Palo Alto, CA, U.S.A.) implanted subcutaneously in the back a few hours after tumour transplantation. MPA was injected daily (5 mg/rat, intramuscularly) for 10 days after the inoculum. Rats adrenalectomized 6 days before tumour transplantation were given 0.9% (w/v) NaCl to drink and, to maintain their blood glucocorticoids within the normal range, a daily intramuscular injection of corticosterone (0.714 mg/kg body wt.) for the whole experimental period (from 6 days before transplantation until 4 or 10 afterwards).

Rats were weighed and anaesthesized with diethyl ether; tumours were harvested from the peritoneal cavity, their volume and cellularity were measured, and blood was collected from the abdominal aorta. Plasma was prepared by centrifuging the whole blood at 3000 rev./min for 15 min at 4 °C and stored in batches at −80 °C. Many organs were removed and weighed, and liver and gastrocnemius muscle were frozen in liquid nitrogen and stored at −80 °C. The lumbar fat-pads were considered representative of the whole white-adipose-tissue mass; the brown adipose tissue was dissected from the interscapular white adipose tissue.

Protein turnover was evaluated as described by Tessitore et al. (1987, 1993a). Animals received a single intraperitoneal dose of [^{14}C]bicarbonate 1 day before tumour transplantation. Liver and gastrocnemius were homogenized to evaluate total and specific (per mg of protein) radioactivity, as previously reported (Baccino et al., 1982; Tessitore et al., 1987). The following equations were used to calculate the fractional rates of protein synthesis (k_s), degradation (k_d) and accumulation (k_a):

$$k_s = \ln(\text{protein specific radioactivity})/t$$

$$k_d = \ln(\text{total protein radioactivity})/t$$

$$k_a = \ln(\text{total protein})/t$$

Reference is made to a previous paper (Tessitore et al., 1987) for a short discussion about the methodology as applied to liver and skeletal muscle.

Blood constituents, hormones, TNF and free amino acids in the gastrocnemius muscle were determined as previously reported (Tessitore et al., 1993a,b).

Tissue proteinase activities were determined in the soluble extract of liver and gastrocnemius muscle obtained by centrifuging the crude homogenate (6×10^6 *g*-min at 4 °C). Total activities of cathepsin B (EC 3.4.22.1), H (EC 3.4.22.16) and L (EC 3.4.22.15) were measured fluorimetrically by the methods described by Barrett (1980) and Barrett and Kirschke (1981), by using synthetic substrates containing 4-methyl-7-coumarylamide (MCA). In particular, carboxybenzoyl-L-arginyl-L-arginyl-MCA, L-arginyl-MCA and carboxybenzoyl-L-phenylalanyl-L-arginyl-MCA were the specific substrates for cathepsins B, H and L respectively. Cathepsin D (EC 3.4.23.5) activity was determined in the presence of 1% (w/v) Triton X-100, by evaluating the hydrolysis of haemoglobin by the method of Barrett (1967).

Data are presented as means ± S.D. (or ± S.E.M. in some Tables). Significance of differences was calculated by ANOVA. As for the fractional rates of protein turnover, comparisons were made on linear regressions (Lee and Lee, 1982).

Chemicals

Insulin zinc-protamine was from Eli Lilly and Co. (Indianapolis, IN, USA), acetylsalicylic acid (ASA) (Flectadol) from Maggioni Farmaceutici (Milano, Italy), leupeptin from Sigma (St. Louis, MO, U.S.A.), MPA (Depo-Provera) from Upjohn (Puurs, Belgium), and $NaH^{14}CO_3$ (53 mCi/mmol) from New England Nuclear (Boston, MA, U.S.A.). All other reagents were from Merck (Darmstadt, Germany).

RESULTS

Body weight, tissue weight and tissue protein

As previously reported (Tessitore et al., 1987), the endoperitoneal growth of the AH-130 tumour was exponential for the first 5 days, then decelerated into a stationary state attained at approx. day 8. The present observations were generally made at two time points: day 4, as representative of the first phase, and day 7 or 10, corresponding to a beginning or fully established last phase. The daily food intake progressively decreased after tumour transplantation from 18 g (day 0) to about 10 g (day 10; cf. Tessitore et al., 1993a). None of the treatments significantly affected this pattern, except INS, which augmented the food intake in tumour hosts to about 20 g at day 10 after implantation.

After tumour transplantation, rats suffered from a progressive severe loss of body weight and of skeletal-muscle protein, most notably from the gastrocnemius, until frank cachexia ensued (Tessitore et al., 1987, 1993a). The data for untreated tumour bearers in Figure 1 and in Tables 2–4 are consistent with those previously reported and further show that brown and, particularly, white adipose tissue were strongly depleted as well. Liver and spleen, after a transient increase in protein content, eventually shared the general pattern of wasting. Only adrenals were still enlarged at day 10.

Different treatments were evaluated on AH-130 tumour-bearing animals. ASA is a cyclo-oxygenase inhibitor that, like indomethacin, has the ability to prevent the acceleration of muscle protein degradation induced by TNF (cf. Tanaka et al., 1989; Gelin et al., 1991) and PGE_2 (cf. Gelin et al., 1991), which are both elevated in AH-130 tumour hosts (Tessitore et al., 1993b). Muscle wasting is frequently associated with increased activity of proteolytic enzymes, and LPP is a well-known inhibitor

Table 1 Growth of the AH-130 hepatoma

Data are means ± S.D. ($n = 6$)

Treatment	10^{-6} × No. of cells per tumour		
	Day 4	Day 7	Day 10
None	2667 ± 168	5261 ± 644	6129 ± 609
ASA	2747 ± 89	4513 ± 687	–
INS	2670 + 55	–	5975 ± 427
LPP	2406 ± 346	–	–
MPA	–	–	6046 ± 670
ADX	2560 ± 91	–	–

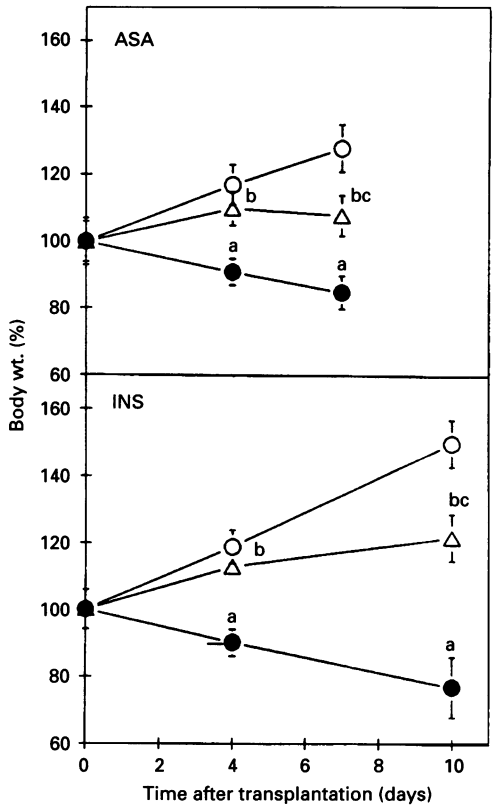

Figure 1 Effect of ASA and INS on body weight (exclusive of the tumour)

Symbols: ○, controls; ●, untreated tumour hosts; △, treated AH-130 bearers. Data are expressed as percentages of controls ($n = 6$). Significance of the differences: $^{a}P < 0.01$, $^{c}P < 0.05$ versus control; $^{b}P < 0.05$ versus untreated tumour hosts.

of cysteine and other proteinases (cf. Tessitore et al., 1988), which has been shown to prevent muscle waste in septic rats (Ruff and Secrist, 1984). The analogue of megestrol acetate, MPA, has been reported to preserve the body weight in breast-cancer patients (Tchekmedyian et al., 1987; Aisner et al., 1988). INS is an anabolic hormone, the plasma level of which was found to be markedly decreased in AH-130 (Tessitore et al., 1993b) or in other tumour hosts (Goodlad et al., 1975; Lanza-Jacoby et al., 1984; Inculet et al., 1987). In addition, since adrenals were found to be enlarged and plasma corticosterone and catecholamines were elevated in AH-130 hosts (Tessitore et al., 1993b), a further group of rats adrenalectomized before tumour inoculation was included.

In non-tumour-bearing rats the present treatments did not cause any appreciable alteration of the parameters evaluated in tumour bearers (results not shown); only INS produced a small increase of liver protein and white-fat mass (cf. Svaninger et al., 1987a). None of the above agents nor ADX affected tumour growth (Table 1). Although cyclo-oxygenase inhibitors such as indomethacin or ASA have been previously reported to inhibit the growth of experimental tumours (Lynch et al., 1978; Gelin et al., 1991), the lack of effects of INS is in keeping with the observation that protein turnover in AH-130 tumour cells is not altered by this hormone (Tessitore et al., 1988).

Treatment with ASA had remarkable effects on tumour bearers at day 4. The loss of body weight (Figure 1) and of gastrocnemius wet weight (Table 2) or protein (Table 4) was significantly attenuated. The enlargement of liver, spleen and adrenals (Table 2) and the increase in liver protein (Table 4) were abolished. The white-adipose-tissue mass was almost completely preserved, whereas the depletion of brown adipose tissue was unaffected (Table 2). However, when the treatment was applied for 7 days, the pattern was clearly different and only the spleen enlargement was still significantly modified (Table 2).

INS counteracted quite effectively the wasting in AH-130 tumour bearers at day 4 as well as at day 10 (Figure 1, Tables 3 and 4). Body weight, tissue weight or protein, and mass of white and brown fat were all maintained close to the control values, and the spleen enlargement at day 4 was abolished; only adrenals at day 10 were even more enlarged than in untreated tumour bearers. In pair-feeding experiments (results not shown), non-tumour-bearing animals either untreated or given INS were allowed to eat the same amount of food consumed by the corresponding groups of tumour bearers. In animals pair-fed and INS-treated for 4 days, (i) the loss of body or gastrocnemius weight did not significantly differ with respect to pair-fed untreated animals, but (ii) the decrease in liver weight was largely prevented and that of white fat was even over-corrected; (iii) in the liver, the protein synthesis rate was increased and the acceleration of protein degradation elicited by food restriction suppressed; (iv) in the gastrocnemius, protein synthesis and degradation rates were both increased, resulting in only minor changes in the net protein balance. Such experiments could not be further extended, however, since pair-fed animals did not survive the INS treatment any longer than 5 days, unlike tumour bearers, which survived at least for the whole experimental period. In agreement with Svaninger et al. (1987a), death in these animals was possibly due to a sudden fall in glycaemia, resulting from the combined direct action of INS and inability to meet the need for substrates by augmenting the food intake or, under the anabolic constraints imposed by INS, by mobilizing protein and lipid stores. Therefore, although the above pair-feeding experiments seem of limited help in discriminating the role played by the increased food intake from the direct effects of the hormone in INS-treated AH-130 hosts, they clearly indicate that AH-130 hosts had developed a significantly higher degree of INS-resistance in comparison with pair-fed rats (cf. Tessitore et al., 1993b).

The loss of liver or gastrocnemius protein in AH-130 hosts was not modified by MPA (Table 4), whereas LPP partially prevented the muscle protein loss at day 4 without affecting the liver enlargement (Table 4). ADX did not significantly modify the decline of body weight in tumour bearers, but slightly increased the weight loss in brown adipose tissue and, particularly,

Table 2 Effect of ASA on tissue wet weight in AH-130 hepatoma-bearing rats

Data are means ± S.D. ($n = 6$). Wet tissue weights are expressed as mg per 100 g initial body wt. For bilateral tissues, values are averages of the two. Significance of the differences (at the same time): *$P < 0.01$ for AH-130 versus controls; †$P < 0.01$, ‡$P < 0.05$ for AH-130 + ASA versus controls; §$P < 0.01$, ‖$P < 0.05$ for AH-130 + ASA versus AH-130.

	Tissue wet wt. (mg/100 g)					
	Day 4			Day 7		
		AH-130			AH-130	
	Controls	−ASA	+ASA	Controls	−ASA	+ASA
Gastrocnemius	602 ± 40 (100)	520 ± 20* (86)	567 ± 27§ (94)	650 ± 10 (100)	465 ± 26* (71)	521 ± 16† (80)
Soleus	50 ± 1.9 (100)	49 ± 1.2 (98)	49 ± 1.3 (97)	48 ± 3.7 (100)	35 ± 4.1* (73)	39 ± 2.9† (82)
Spleen	386 ± 30 (100)	638 ± 45* (165)	394 ± 93§ (102)	325 ± 35 (100)	494 ± 99* (152)	381 ± 48‡‖ (117)
Kidney	366 ± 9 (100)	342 ± 30 (93)	343 ± 22 (94)	404 ± 25 (100)	377 ± 52 (93)	383 ± 52 (95)
Liver	4085 ± 90 (100)	4419 ± 87* (108)	3936 ± 99§ (96)	4395 ± 194 (100)	4825 ± 193 (110)	4565 ± 220 (104)
Adrenal	8.2 ± 0.3 (100)	10.3 ± 0.7* (125)	7.8 ± 0.9§ (95)	9.4 ± 0.9 (100)	11.3 ± 1.1* (120)	11.3 ± 3.2† (120)
White fat	526 ± 17 (100)	324 ± 19* (62)	487 ± 20§ (93)	548 ± 22 (100)	98 ± 6* (18)	–
Brown fat	111 ± 10 (100)	91 ± 8* (82)	88 ± 7† (79)	135 ± 12 (100)	74 ± 5* (55)	–

Table 3 Effect of INS on tissue wet weight in AH-130 hepatoma-bearing rats

Data presentation is as in Table 1. Significance of the differences: *$P < 0.01$ for AH-130 versus controls; †$P < 0.01$, ‡$P < 0.05$ for AH-130 + INS versus controls; §$P < 0.01$, ‖$P < 0.05$ for AH-130 + INS versus AH-130 ($n = 6$).

	Tissue wet wt. (mg/100 g)					
	Day 4			Day 10		
		AH-130			AH-130	
	Controls	−INS	+INS	Controls	−INS	+INS
Gastrocnemius	626 ± 24 (100)	539 ± 29* (86)	611 ± 27§ (98)	849 ± 30 (100)	454 ± 51* (53)	674 ± 63†§ (79)
Soleus	44 ± 2.6 (100)	43 ± 1.1 (100)	45 ± 3.1 (103)	57 ± 2.2 (100)	42 ± 5.3* (74)	53 ± 5.0§ (93)
Spleen	430 ± 60 (100)	521 ± 17* (121)	429 ± 14§ (100)	503 ± 27 (100)	245 ± 18* (49)	543 ± 37‡§ (108)
Kidney	390 ± 25 (100)	381 ± 20 (98)	379 ± 23 (97)	503 ± 29 (100)	409 ± 22* (81)	469 ± 46‖ (93)
Liver	4552 ± 138 (100)	4944 ± 189* (109)	4914 ± 150† (108)	5660 ± 287 (100)	3946 ± 20* (70)	5678 ± 76§ (100)
Adrenal	9.2 ± 0.9 (100)	10.9 ± 0.8* (119)	10.3 ± 1.5‡ (112)	11.0 ± 0.4 (100)	14.4 ± 0.8* (131)	17.3 ± 2.2†§ (157)
White fat	496 ± 17 (100)	359 ± 49* (51)	444 ± 52‖ (72)	541 ± 20 (100)	84 ± 8* (16)	600 ± 33§ (111)
Brown fat	134 ± 11 (100)	108 ± 10* (81)	130 ± 15‖ (97)	163 ± 18 (100)	76 ± 13* (47)	134 ± 27§ (82)

produced a most severe depletion of white fat since day 4 (results not shown).

Blood plasma constituents

In AH-130 tumour hosts the development of cachexia was associated with pronounced alterations of blood plasma constituents; although glucose was normal (cf. Tessitore et al., 1993b), total cholesterol and, even more, triacylglycerols were markedly increased (Dessi et al., 1992). As shown in Table 5, the elevation of triacylglycerols was largely suppressed by INS administration, whereas cholesterol was not significantly affected and glucose was decreased at day 10. Similar changes in the lipid pattern and decreased glucose were observed in animals subjected

Table 4 Effect of treatments on liver and gastrocnemius protein content

Data (mg of protein/100 g initial body weight) are means ± S.D. ($n = 6$). Significance of the differences: *$P < 0.01$ versus control; †$P < 0.01$ versus untreated tumour hosts.

Tissue		Treatment	Protein Day 4	Protein Day 10
Liver	Controls	None	960 ± 50	1194 ± 73
	AH-130	None	1091 ± 60*	832 ± 49*
		LPP	1026 ± 44*	–
		INS	1104 ± 81*	1297 ± 68†
		ASA	956 ± 59†	–
		MPA	–	888 ± 53*
		ADX	940 ± 48†	893 ± 44*
Muscle	Controls	None	102 ± 7	138 ± 10
	AH-130	None	80 ± 7*	64 ± 6*
		LPP	112 ± 11†	–
		INS	108 ± 9†	125 ± 8†
		ASA	96 ± 3†	–
		MPA	–	74 ± 5*
		ADX	90 ± 4*	78 ± 6*

Table 5 Plasma constituents in tumour hosts after ADX or treatment with INS and ASA

Data (mg/dl) are means ± S.D. ($n = 6$). Significance of the differences: *$P < 0.01$, †$P < 0.05$ versus controls; ‡$P < 0.01$ versus untreated tumour hosts at the same time.

	Treatment	Content (mg/dl) Day 0	Day 4	Day 10
Glucose	None	181 ± 14	178 ± 18	170 ± 9
	INS	–	166 ± 12	143 ± 9*
	ADX	–	125 ± 7*	–
	ASA	–	199 ± 10	–
Triacylglycerols	None	95 ± 8	290 ± 25*	246 ± 19*
	INS	–	155 ± 16*‡	145 ± 11*‡
	ADX	–	114 ± 10‡	–
	ASA	–	296 ± 28	–
Cholesterol	None	58 ± 6	118 ± 6*	100 ± 7*
	INS	–	108 ± 6*	94 ± 5*
	ADX	–	115 ± 8*	–
	ASA	–	70 ± 7‡	–

to ADX before tumour transplantation. By contrast, ASA had no influence on the elevation of triacylglycerols, but strongly attenuated that of cholesterol.

As for plasma hormones (Table 6), corticosterone was decreased to control levels by treatment with INS or LPP, but not with ASA, whereas INS remained low in all cases, except for animals receiving this hormone; in non-tumour-bearers corticosterone was not affected by any of these agents (results not shown). The plasma level of TNF, elevated in tumour bearers (cf. Tessitore et al., 1993b), was not significantly modified by INS, ADX or ASA (Table 6). The lack of effects of ASA on TNF levels may be consistent with its production by the tumour rather than by reactive cells (Tessitore et al., 1993b).

Tissue protein turnover

To assess how the tissue protein changes in tumour hosts were

Table 6 Effect of treatments on plasma hormones

AH-130 hosts were used 4 days after tumour transplantation. Data are means ± S.D. ($n = 6$); n.d., not detectable. Significance of the differences: *$P < 0.01$ versus controls; †$P < 0.01$ versus untreated tumour hosts.

Animals	Treatment	Insulin (μ-units/ml)	Corticosterone (mg/dl)	TNF (pg/ml)
Controls	None	78 ± 14	21 ± 5	n.d.
AH-130 hosts	None	25 ± 10*	39 ± 3*	110 ± 12
	ASA	29 ± 13*	41 ± 3*	120 ± 9
	INS	69 ± 17†	21 ± 4†	140 ± 12
	LPP	20 ± 8*	24 ± 6†	–
	ADX	–	–	90 ± 11

Table 7 Effects of treatments on liver protein turnover

The time interval after tumour transplantation is shown in days. Fractional rates are expressed as %/day. Significance of the differences: *$P < 0.05$ versus controls; †$P < 0.05$ versus untreated tumour hosts (for details see the Experimental section).

Animals and treatment	Time interval (days)	k_s	k_d	k_a
Controls				
None	0–4	30	26	4.71
	4–10	16	13	4.84
AH-130 hosts				
None	0–4	32*	22*	7.59*
	4–10	17	23*	−5.86*
INS	0–4	36*†	23	8.43*
	4–10	24*†	21*	3.71†
ADX	0–4	29	34*†	2.03†
ASA	0–4	28*	23	3.94†

modified by the above treatments, bulk protein turnover was evaluated in the liver and gastrocnemius by measuring protein radioactivity decay starting 24 h after labelling with $NaH^{14}CO_3$. As discussed previously (Tessitore et al., 1987), this approach to investigate protein turnover *in vivo* is very suitable for the liver (Swick and Ip, 1974), but it is affected by some degree of label recycling in skeletal muscle (MacDonald et al., 1979). Two observation windows were adopted, corresponding to days 0–4 and days 4–10 after tumour transplantation. In liver, though not in muscle, protein turnover rates in these intervals were quite different, due to the heterogeneous half-lives of proteins of the slow-turnover pool (Garlick et al., 1975); thus comparisons among the experimental groups should only be made within the same interval.

Previous data from our laboratory (Tessitore et al., 1987) have shown that the transient liver hyperplasia and the subsequent waste were associated with first decreased and then increased rates of protein degradation (k_d), whereas synthesis rates (k_s) were little affected (Table 7). Gastrocnemius protein depletion resulted from enhanced breakdown, with no changes in synthesis rates (Table 8). In adrenalectomized tumour bearers the liver k_d was augmented in the interval days 0–4 (Table 7), whereas gastrocnemius protein turnover was not modified (Table 8); this is consistent with the abrogation of the transient liver hyperplasia, as well as with the lack of effects on muscle waste (Table 4). The enhancement of liver k_s (Table 7) and that of muscle k_d (Table 8)

Table 8 Effects of treatments on gastrocnemius protein turnover

Data presentation is as in Table 7.

Animals and treatment	Time interval (days)	k_s	k_d	k_a
Controls				
None	0–4	12	4.96	4.21
	4–10	12	5.03	5.76
AH-130 hosts				
None	0–4	12	14*	−1.86*
	4–10	11	14*	−2.40*
INS	0–4	15*†	11†	5.13†
	4–10	17*†	15*	2.44†
ADX	0–4	13	16*	0.78*
	4–10	12	16*	−2.39*
ASA	0–4	12	11†	2.23†

Table 9 Free amino acids in gastrocnemius muscle

Data (μmol/g wet wt. of tissue) are means ± S.E.M. ($n = 6$). Significance of the differences: *$P < 0.001$ versus controls; † $P < 0.001$ for AH-130 + INS versus AH-130; ‡ $P < 0.05$ for AH-130 + INS versus AH-130. Abbreviation: AABA, α-aminobutyric acid.

	Controls	AH-130 hosts (day 10)	AH-130 hosts + INS (day 10)
AABA	0.08 ± 0.001	0.08 ± 0.001	0.09 ± 0.001*
Ala	7.75 ± 0.31	7.08 ± 0.25	7.71 ± 1.27
Arg	0.82 ± 0.10	0.87 ± 0.05	0.99 ± 0.01
Asn	1.17 ± 0.02	0.96 ± 0.01*	0.54 ± 0.03*†
Asp	0.39 ± 0.03	0.14 ± 0.01*	0.13 ± 0.01*†
Cit	0.70 ± 0.05	0.36 ± 0.03*	0.66 ± 0.01
Cys	–	–	–
Gln	22.35 ± 1.36	7.44 ± 0.51*	6.93 ± 0.16*
Glu	4.59 ± 0.19	2.97 ± 0.21*	3.48 ± 0.6
Gly	11.01 ± 0.20	5.73 ± 0.11*	10.77 ± 0.31†
His	0.72 ± 0.01	0.69 ± 0.01	0.57 ± 0.01*†
Ile	0.66 ± 0.004	0.84 ± 0.11	0.45 ± 0.03*‡
Leu	0.98 ± 0.02	1.02 ± 0.08	0.66 ± 0.03*†
Lys	1.70 ± 0.12	1.92 ± 0.07	2.34 ± 0.24
Met	0.42 ± 0.01	0.48 ± 0.01	0.30 ± 0.001†
Orn	0.20 ± 0.001	0.17 ± 0.001	0.17 ± 0.01
Phe	0.57 ± 0.01	0.60 ± 0.01	0.39 ± 0.001†
Pro	1.30 ± 0.04	1.20 ± 0.06	1.17 ± 0.01*
Ser	3.04 ± 0.13	2.13 ± 0.11*	2.70 ± 0.27
Tau	39.51 ± 1.21	30.45 ± 0.94	39.09 ± 0.82†
Thr	3.25 ± 0.12	1.77 ± 0.4*	2.34 ± 0.04†*
Trp	0.36 ± 0.02	0.30 ± 0.01	0.21 ± 0.001‡
Tyr	0.75 ± 0.01	0.60 ± 0.01	0.48 ± 0.03‡
Val	1.46 ± 0.03	1.41 ± 0.004	0.72 ± 0.03*†
Total	104.31 ± 1.93	69.22 ± 2.62*	82.90 ± 3.47§*

over days 0–4 were both attenuated by ASA, thus explaining how the initial hepatic hyperplasia was prevented and the muscle waste decreased (Tables 2 and 4). Among the treatments tested, INS was again revealed as the most effective, increasing the k_s in liver (Table 7) and muscle (Table 8) over both observation windows and decreasing the k_d in muscle over days 0–4. The increase in gastrocnemius free amino acids at day 10 (Table 9) is consistent with the improved balance between protein synthesis and degradation (cf. Tessitore et al., 1993a) and the protection afforded by INS on muscle protein (Tables 3 and 4).

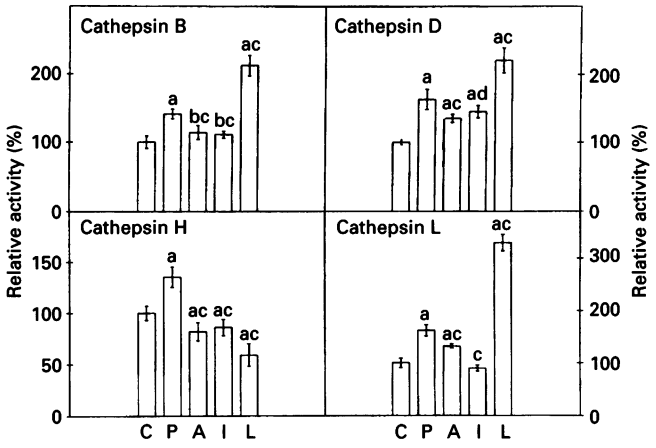

Figure 2 Relative activity of lysosomal proteinases in the liver of tumour-bearing rats after drug treatments

Key: C, controls; P, tumour hosts; A, tumour hosts + ASA; I, tumour hosts + INS; L, tumour hosts + LPP. Significance of the differences: $^aP < 0.01$, $^bP < 0.05$ versus control; $^cP < 0.01$, $^dP < 0.05$ versus AH-130 ($n = 6$).

Tissue proteinases

Since an increased activity of tissue proteinases such as cathepsin D (Lundholm et al., 1980; Greenbaum and Sutherland, 1983; Tessitore et al., 1993a) has been suggested as a possible explanation for the accelerated tissue protein breakdown in tumour-bearing animals, we examined the effects of the above treatments on various lysosomal proteinase activities. In the liver of AH-130 tumour bearers at day 4 the activities of cathepsins B, D, H and L were indeed significantly augmented on a tissue-protein basis (Figure 2), whereas both INS and ASA caused a significant decrease. A different pattern was observed after LPP, with only cathepsin H activity being decreased while that of cathepsins B, D, and L was further and sharply increased; this latter effect likely resulted from inhibition of intralysosomal enzyme breakdown. Only cathepsin D activity was found to be increased (by 55%) in the muscle at day 4, and no activity was modified by any agent tested (results not shown). It is worth noting, however, that proteolytic pathways other than the lysosomal one may be involved in the enhancement of muscle protein breakdown in AH-130 tumour-bearers (Attaix et al., 1992; Baracos et al., 1992).

DISCUSSION

In rats into which the ascites hepatoma Yoshida AH-130 was transplanted (Tessitore et al., 1987, 1993a,b), cachexia is characterized by early onset, rapid progressivity, and a general pattern of tissue waste that particularly involves skeletal muscle and adipose tissue. In previous work, the metabolic competition by the tumour (Tessitore et al., 1987, 1993a,b) or the progressive decline in food intake (Tessitore et al., 1993a,b) did not appear to play a primary role in host wasting; rather, the dominant emerging feature was a hypercatabolic setting of tissue metabolism enforced through humoral mediation, and that should be viewed as cause rather than consequence of the waste (Tessitore et al., 1993b; Costelli et al., 1993). A definite shift in the

hormonal profile towards a catabolic pattern, increased plasma PGE_2 and presence of circulating TNF, as detected in AH-130 tumour-bearing animals (Tessitore et al., 1993b), could well account for such a mediation; but other factors such as γ-interferon (Matthys et al., 1991), leukaemia-inhibitory factor (Mori et al., 1991) or interleukin-6 (Greenberg et al., 1992) could play a role as well. It thus seemed of interest to examine whether pharmacological and hormonal interventions liable to interfere at various levels with the enhanced tissue protein degradation or other metabolic derangements could significantly prevent or attenuate tissue waste (Ruff and Secrist, 1984; Kettelhut et al., 1987; Moley et al., 1988). Of interest, none of the present treatments significantly affected tumour growth or the circulating levels of TNF.

In spite of the sustained elevation of plasma corticosterone and catecholamines in AH-130 tumour hosts (Tessitore et al., 1993b), ADX did not substantially alter the general course of cachexia, in agreement with previous findings (Svaninger et al., 1987b). By contrast, a partial but quite evident prevention of body-weight loss and muscle wasting was afforded by ASA at day 4 after tumour transplantation, though no longer at day 7; such effects may well be accounted for by the ability of this drug to interfere with the peripheral action of PGE_2 or TNF, both of which are elevated in AH-130 tumour hosts (Tessitore et al., 1993b). Likewise, muscle protein loss in AH-130 tumour hosts was partially prevented by the proteinase inhibitor LPP, probably via direct interference with the final proteolytic steps in protein degradation. LPP also abolished the elevation of plasma corticosterone in AH-130 bearers, while not affecting plasma INS. Although the effects of glucocorticoids on muscle protein turnover remain controversial (Odedra and Millward, 1984; Sugden and Fuller, 1991), the present data do not rule out the possibility of an aggravating role for these hormones in tissue waste. Thus a possible indirect effect of LPP in this way cannot be ruled out.

Among the agents tested, INS proved the most effective on AH-130 tumour hosts at a daily dosage that was adequate to restore normal plasma INS levels (whereas corticosterone was not affected). Body weight, tissue protein content or turnover, adipose tissues and plasma lipids all showed marked and sustained improvements, likely reflecting the pleiotropic action of INS as well as the increased food intake (cf. Morrison, 1982; Moley et al., 1985, 1988; Chance et al., 1986; Peacock et al., 1987; Rofe et al., 1989). Tissue protein was preserved by INS through increased synthesis rates in the liver and gastrocnemius. Moreover, an appreciable decrease in breakdown rates was observed in the muscle, at least in the first observation window (days 0–4). Consistently, muscle total free amino acids were close to the control levels (cf. Chance et al., 1986). These observations are in agreement with previous reports, wherein INS was shown to restore a positive nitrogen balance and to prevent the body-weight loss in mice bearing the MAC16 colon adenocarcinoma (Beck and Tisdale, 1989) or to preserve the body weight of sarcoma-bearing rats, though not prolonging their survival (Morrison, 1982; Moley et al., 1985, 1988); moreover, whole-body protein turnover was improved by INS in cancer patients, however insulin-resistant they were with respect to glucose metabolism (Schein et al., 1979; Heslin et al., 1992). Moreover, in spite of this marked anti-cachectic action, INS did not antagonize all of the metabolic perturbations in AH-130 hosts, such as, in particular, the elevation of plasma total cholesterol. This was suppressed by ASA, however, suggesting a role for some ASA-suppressible mechanism in the increase of total cholesterol. By contrast, elevation of triacylglycerols, though not affected by ASA, was suppressed by ADX or INS; since the latter agent also re-established a normal level of corticosterone, a role for this hormone in the increase in triacylglycerols can be postulated.

The present data further support the view (Tessitore et al., 1993b) that the onset of cancer cachexia in AH-130 tumour hosts is related to the interplay of a complex network of factors that altogether concur in determining tissue waste by imposing a hypercatabolic state (cf. Lowry, 1991). Treatment with anti-TNF antibodies (Costelli et al., 1993; N. Carbó, P. Costelli, L. Tessitore, G. J. Bagby, F. J. Lopez-Soriano, F. M. Baccino and J. M. Argilés, unpublished work) or with such diverse agents as ASA, LPP, or, particularly, INS, as shown in the present work, all afforded a significant protection by interfering with proximal or distal processes in such waste. The administration of INS has been advocated to improve the nutritional state of weight-losing tumour-bearing individuals (cf. Schein et al., 1979; Morrison, 1982; Chance et al., 1986; Peacock et al., 1987; Moley et al., 1988; Rofe et al., 1989). In the present work, INS treatment restored a normal food intake in tumour bearers, which is likely to have concurred significantly in antagonizing the development of cancer cachexia. In view of the well-known observation that overfeeding is of quite limited benefit to cancer patients (Brennan, 1981; Bozzetti et al., 1987; Shaw and Wolfe, 1988), such an effect can hardly provide by itself a full explanation for the anti-cachectic action of INS. The data thus far available do not permit us to discriminate the respective contribution of these two mechanisms in the anti-cachectic action of INS on AH-130 hosts. Generally speaking, however, our previous and current observations on the present cachexia model lead us to share the view expressed by Heslin et al. (1992), on the basis of clinical studies, that hormonal manipulations using anabolic agents such as INS may be expected to play a significant role in the management of cancer individuals. Further studies thus seem warranted in the search for drugs having the ability to interfere proximally or distally with the humoral mediation of cachexia. Along these lines, a note of caution is necessary, however, since such agents might also have the potential to accelerate tumour growth, either promoting cell proliferation or preventing cell death.

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