epithelium. On an earlier occasion, an equivalent daily dosage of indomethacin has been observed to inhibit the healing of skin wounds in the rat[2]. The difference in results could be explained, for example, by the fact that the external surroundings of a skin wound are completely different from those of an extraction wound.

A 5-days' indomethacin treatment retarded the ossification of the alveolar socket to a certain extent. Perhaps indomethacin has some effect on the function of osteoblasts and osteoclasts. Further studies are required for resolving this problem.

Zusammenfassung. Untersuchungen an der Ratte zeigten, dass nach Zahnextraktionen die Zufuhr von Indomethazin ein postoperatives Oedem beträchtlich reduziert, dabei aber die Ossifikation der Alveolenhöhle verzögert.

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Inhibition of Adriamycin Cardiotoxicity by Acetyldaunomycin

Therapy with the anthracycline antibiotics daunomycin (D) and adriamycin (A) has frequently been associated with cardiomyopathy and congestive heart failure[1–10]. The cardiotoxic effects may be recognized early by careful monitoring of heart functions[11]. But the only practical measure to protect the patients is a strict limitation of the total dose to approximately 500 mg/m² [10].

Various approaches have been tried to reduce cardiotoxicity of anthracycline antibiotics. In acute experiments, mecamylamine, hexamethonium, guanethidine, and reserpine were shown to inhibit arrhythmia induced by i.v. infusions of D into hamsters[12]. Moreover, the chelating agents EDTA and ICRF 159 were found to counteract the increase in coronary perfusion pressure caused by D and A in the isolated dog heart[13]. A clinical application of these pharmacological observations was not tried.

Cardiotoxicity could also be reduced by administering the antibiotics in the form of DNA complexes. These are thought to be incorporated into the cells by pinocytosis, avoiding high free drug concentrations in the extracellular space[14]. Encouraging support for this concept was provided by preliminary clinical observations[15].

The present attempts to reduce cardiotoxicity of A were motivated by pharmacokinetic and toxicological observations: After i.v. injections into hamsters, a large proportion of anthracycline antibiotics were taken up by various tissues, including the heart. Within 5 min a daunomycinone-like aglucone was found in animals treated with D and A, reaching levels in the heart of 12% and 37% of the injected dose respectively. With acetyl daunomycin (AD), a drug which is N-acetylated at the sugar (daunosamine) moiety, the aglycone was found only after 30 min. Its levels never exceeded 2% of the injected dose[16]. After repeated i.p. injection in rats, the first signs of cardiotoxicity, changes of the QRS complex in the electrocardiogram (ECG), were observed with a cumulative dose of 80 mg/m² of D and 68 mg/m² of A[17]. Similar doses also caused disturbances of the metabolism of heart mitochondria[18]. AD, however, caused no significant changes of the ECG and mitochondrial metabolism at cumulative doses exceeding 800 mg/m² [17,18].

Electron microscopic studies demonstrated a direct injurious effect of D and A on mitochondria and myofibrils of the heart[19]. Since AD readily entered the heart muscle[16] but was not cardiotoxic, it was conceivable that it might compete with A and D for binding sites on structural proteins and thereby protect myocardial cells against the injurious effects of the more toxic derivatives. The experiments reported in this paper bear out this hypothesis.

Materials and methods. Groups of 6 female rats of the SIV-50 strain weighing approximately 160 g were injected i.p. 5 times weekly with A, AD, or A plus AD as indicated

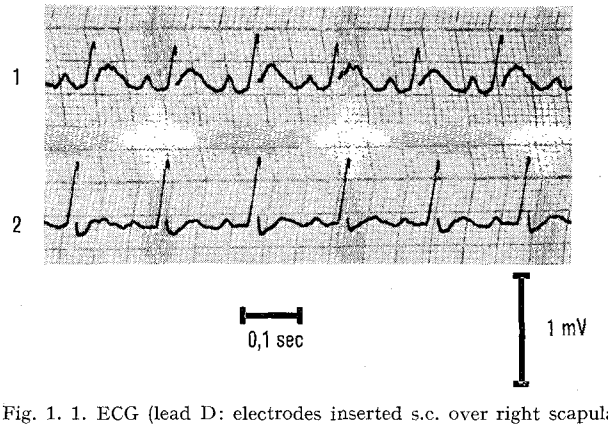

Fig. 1. 1. ECG (lead D: electrodes inserted s.c. over right scapula and sacral vertebrae) of unanesthetized, untreated rat. 2. ECG (lead D) of the same rat, after 6 injections of 4 mg/kg A. Marked widening of QRS with appearance of a distinct S-wave trough. Moderate flattening of T-wave and bradycardia.

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in the Table. A was provided in 10 mg vials containing 50 mg lactose, AD as pure substance. Solutions were made fresh daily with 0.9% NaCl, to give an injected volume of 0.2 ml/100 g body weight. ECGs were recorded before treatment and 2 to 3 times weekly thereafter, as described previously[17]. White blood cell counts (WBC) were performed once weekly using a Coulter Counter Model B. Surviving animals were sacrificed on the 36th day and inspected for presence of ascites. Hearts were excised, weighed, and fixed in Bouin's fluid. Atrial and ventricular tissues were embedded in paraffin, sectioned and stained with hematoxylin-eosin.

Results. The typical early ECG changes induced by cardiotoxic anthracycline antibiotics are demonstrated in Figure 1: Widening of the QRS complex develops first; it is usually followed by the appearance of a distinct S-wave trough. Flattening of the T-wave, bradycardia, sometimes also intraventricular block and atrial fibrillation may be found in later stages[17].

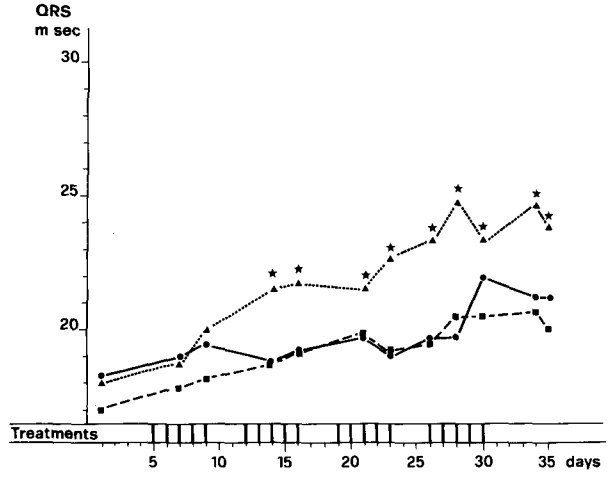

Fig. 2. QRS in ECGs of rats treated 5 times weekly with: ▲.....▲, A 1 mg/kg; ■– – – –■, AD 4 mg/kg; ●——●, A 1 mg/kg plus AD 4 mg/kg. Mean of groups of 6 rats. *Statistically significant difference ($p < 0.05$, U-test of Mann and Whitney) compared to 1 mg/kg A in combination with 4 mg/kg of AD.

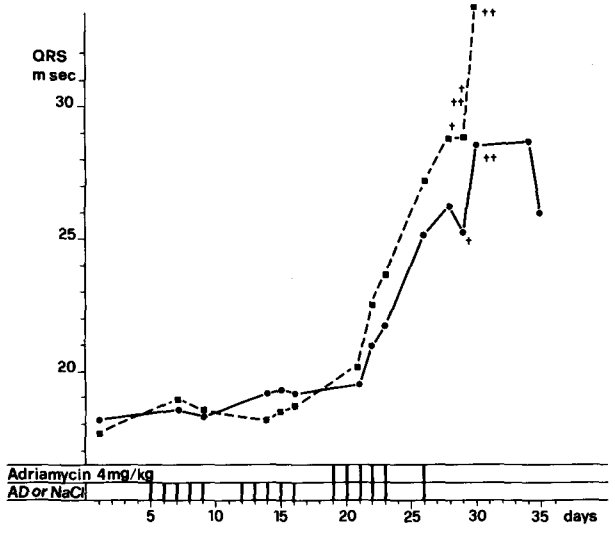

Fig. 3. QRS duration in ECGs of rats treated with: ■– – – –■, 2 ml/kg 0.9% NaCl for 2 weeks, followed by 6 injections of 4 mg/kg A plus 2 ml/kg 0.9% NaCl; ●——●, 4 mg/kg AD for 2 weeks followed by 6 injections of 4 mg/kg A plus 4 mg/kg AD. †, death of 1 rat.

For quantitative evaluation of the cardiotoxic effects measurement of the QRS duration plus S-wave trough has proved to be the most reliable parameter[17]. Figure 2 shows the results obtained in rats treated with 1 mg/kg A with and without concomitant treatment with 4 mg/kg AD, and in animals receiving 4 mg/kg AD. A caused a steady widening of the QRS complex with development of a S-wave trough. Only a very slight widening of the QRS complex was observed in the groups treated with A plus AD or AD alone. The difference between these groups and that treated with A alone was statistically significant from the 8th treatment day to the end of the experiment. Body weight gain and WBC were reduced in the animals treated with A with and without AD, but there was no statistically significant difference between these two groups (Table). Two additional groups of rats were pretreated with 4 mg/kg of AD 5 times weekly for 2 weeks or equal volumes of 0.9% NaCl. Both groups then received 6 injections of 4 mg/kg A plus 4 mg/kg AD or equal volumes of 0.9% NaCl. In the saline pretreated group rats commenced to die on the second day after the last injection of A and all were dead 3 days later. With AD pretreatment 3 animals died 4–6 days after the last A injection and 3 survived. Widening of the QRS complex was more pronounced in the saline pretreated rats, but weight loss and leukopenia were identical in both groups (Figure 3 and Table).

At autopsy of the surviving animals, ascites was noted in half of the rats treated with 1 mg/kg of A but not in the groups receiving 4 mg/kg AD with or without 1 mg/kg A. There were no significant differences in absolute and relative heart weights.

The histopathological evaluation of the myocardial tissue revealed only modest changes in part of the animals. There were small foci of fibre atrophy with vacuolization and slight increase in interstitial tissue. These alterations were most frequently seen in the group treated with saline plus 4 mg/kg A, and least frequently in rats receiving 4 mg/kg AD. But the lesions were not well developed and could thus not be evaluated quantitatively.

Discussion. The biochemical basis of the antitumor action of anthracycline antibiotics is a complex formation with DNA, leading to inhibition of DNA replication and DNA-dependent RNA synthesis[20, 21]. This effect is also responsible for bone marrow suppression, as demonstrated in our experiments by a drop in WBC. AD had no effect on the leukopenic action of A. This was not expected, since AD is known to inhibit the growth of certain experimental tumors[22] and thus probably affects DNA in the same manner as A.

Experimental evidence indicates that anthracycline antibiotics also affect other cellular macromolecules. For example, heart mitochondrial structure[8, 10, 19, 23] and function[18] were rapidly and markedly impaired by A, D, and other cardiotoxic derivatives. Moreover, structural

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General toxicity, body weight changes and leukopenic effects of adriamycin, acetyldaunomycin and combinations

Group	Treatment [a]	Number of deaths	Mean body weight changes during treatment (g)	Maximal decrease in WBC during or after treatment (% of pretreatment values)
I	AD 4 mg/kg, 4 weeks	0/6	70.2	21
II	A 1 mg/kg, 4 weeks	0/6	32.5 [b]	44.5 [b]
III	AD 4 mg/kg plus A 1 mg/kg, 4 weeks	0/6	34.3 [b]	31.8
IV	2 ml/kg 0.9% NaCl, 2 weeks, followed by 6 × 4 mg/kg A plus 2 ml/kg NaCl	6/6	−20.8	86
V	AD 4 mg/kg, 2 weeks, followed by 6 × 4 mg/kg A plus 4 mg/kg AD	3/6	−19.0	80

[a] Five times weekly by i.p. injection. A, adriamycin; AD, acetyldaunomycin. [b] Statistically significant difference ($p < 0.05$, U-test of Mann and Whitney) compared to group I.

changes of myofibrils comparable to those induced by other cardiotoxic chemicals, were observed in D- and A-treated animals and in patients dying in congestive heart failure after therapy with anthracycline antibiotics[8, 10, 19]. It is probable, therefore, that the cardiomyopathy induced by these drugs is, at least in part, due to a lesion of structural proteins.

Earlier experiments have shown that AD was much less cardiotoxic than D and A: after i.v. infusion into Syrian golden hamsters and rhesus monkeys AD did not ellicit cardiac arrhythmias as seen with the other 2 drugs[24]. In rats, daily i.p. injections of doses as high as 8 mg/kg did not cause significant ECG changes[17] or impairment of heart mitochondrial metabolism[18]. This lack of cardiac toxicity might be due to the very low rate of aglycone formation in tissues[16]. Since AD accumulated in the heart as efficiently as D and A it is conceivable that it is bound to the same biological structures as the 2 toxic derivatives. When given at high enough doses it may thus effectively compete for binding sites and antagonize the toxic effects of related drugs. The present experiments provide good evidence that this mechanism is indeed possible. 20 doses of 1 mg/kg A did not induce significant ECG changes in rats when administered together with a 4-fold higher dose of AD. When the same A dose was injected with equal volumes of saline, definite ECG changes occurred towards the end of the second week of treatment, confirming 5 previous experiments using the same technique ([17] and unpublished experiments). Ascites was present in half of the rats treated with 1 mg/kg A but was not observed in the group receiving the same dose of A together with 4 mg/kg AD[25].

A slight antagonistic effect was also observed when 4 mg/kg of AD were given 2 weeks before and together with 6 doses of 4 mg/kg of A. With 4 mg/kg A alone, marked ECG changes developed rapidly and all rats died shortly after treatment. In combination with AD, ECG changes were not as marked and only half of the animals died. The histopathological changes of the myocardial tissues were not very pronounced and could therefore not be used to confirm the antagonistic effect of AD.

The reduction of the cardiotoxic effect of A by AD is of sufficient interest to be investigated further. Unfortunately, like most semisynthetic anthracycline antibiotics, AD is in short supply. The experiments presented in this paper indicate, however, that additional anthracycline antibiotics should be developed in order to find compounds with a high affinity to myocardial tissue but without adverse biological effects. Such derivatives, even if they had no chemotherapeutic action, could then be used in conjunction with highly active compounds whose cardiotoxicity they may reduce to a significant degree.

Zusammenfassung. Die kardiotoxische Wirkung von Adriamycin wurde im Elektrokardiogramm von Ratten nach wiederholten i.p. Injektionen nachgewiesen. Bei gleichzeitiger Behandlung mit Acetyldaunomycin waren die elektrokardiographischen Veränderungen signifikant geringer. Die letale Wirkung grosser Adriamycindosen wurde durch Acetyldaunomycin ebenfalls abgeschwächt. Dagegen hatte Acetyldaunomycin keinen Einfluss auf die durch Adriamycin erzeugte Leukopenie.

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