Anti-kanzerogene Wirkung von *cis*-Aconitsäure bei Mäusen

Substanz	Injizierte Menge in mg/0,5 ml Tricaprylin		Zahl der Tiere[a]	Zahl der Tiere gestorben innerhalb 7 Monaten nach 3,4-BP Injektion		Zahl der Tiere gestorben nach 7 Monaten		Zahl der noch lebenden Tiere[b]		3,4-Benzopyren Kanzerogenese (%)
	3,4-BP (mg)	Zusatz (mg)		Mit Tumor	Ohne Tumor	Mit Tumor	Ohne Tumor	Mit Tumor	Ohne Tumor	
Kontrolle (ohne Behandlung)	—	—	28 (2)	—	—	—	—	—	28 (340)	0
Nur 3,4-Benzopyren Gruppe A	2,52	—	30	30	—	—	—	—	—	100
3,4-BP+cis-Aconitsäure Gruppe D	2,52	< 10 (gesätt.)	35	23	—	4	8	—	—	77
3,4-BP+cis-Aconitsäure Gruppe C	2,52	10 (+ Äthan.)	19 (1)	10	—	2	7	—	—	63
3,4-BP+cis-Aconitsäure Gruppe B	2,52	30 (+Äthan.)	34	3	2	3	1	—	25 (340)	18

Abnahme der 3,4-BP Kanzerogenese von 100% auf 18% bei steigender *cis*-Aconitsäure Konzentration 0–30 mg/2,52 mg 3,4-BP. [a] In Klammern Zahl der Mäuse gestorben unmittelbar nach der Behandlung durch Unfälle, Krankheiten, Kannibalismus, Toxizität. [b] Beobachtungszeit in Tagen.

Resultate und Diskussion. Bei Mäusen die nur mit 2,52 mg 3,4-BP behandelt wurden, war die Tumorentwicklung 100%. Eine Abnahme der Tumorbildung bis 18% wurde in Abhängigkeit von der steigenden *cis*-Aconitsäure Konzentration festgestellt. Es ist noch die Frage zu klären, ob eine vollständige Hemmung der 3,4-BP Kanzerogenese durch noch höhere *cis*-Aconitsäure-Konzentrationen möglich wäre. Es wurde ausserdem zum Teil eine Verzögerung der Tumorbildung durch *cis*-Aconitsäure beobachtet.

Die bisher durchgeführten Untersuchungen deuten darauf hin, dass ein biologischer Inaktivierungsmechanismus der kanzerogenen Wirkung von 3,4-Benzopyren (und eventuell anderen kanzerogenen Stoffen) unter anderem durch Stoffwechselprodukte, die in einigen Organismen physiologisch vorkommen, wie z.B. *cis*-Aconitsäure oder durch Substanzen, die in ihrem Verdauungstrakt durch Bakterien gebildet sind, z.B. Putrescin, theoretisch denkbar wäre.

Summary. The cancerogenic action of 3,4-Benzopyrene (3,4-BP) can be reduced by several natural and synthetic compounds. The addition of 10–30 mg of *cis*-aconitic acid to 2.52 mg 3,4-BP can considerably decrease the incidence of tumor development. Since *cis*-aconitic acid is a metabolic product of the citric acid cycle, its significance for the biological inactivation of the cancerogenic action of 3,4-BP is theoretically possible.

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[3] Herrn Dr. E. FASSKE danke ich für die histologische Untersuchung der Tumoren.

Inhibition of Mucous Metaplasia in the Skin Tumor Keratoacanthoma by Continual Applications of Puromycin

The keratoacanthoma is a rapidly growing, dry skin tumor which undergoes excessive keratinization and can be found in man[1] and can be produced in laboratory animals[2–8]. Previous studies have demonstrated that when the experimental keratotic tumor is treated with topical applications of vitamin A acid, a change in the epithelial product of tonofibrils to mucus results[4].

The purpose of the present investigation was to observe, at macroscopical and ultramicroscopical levels, the effect of puromycin on the action of vitamin A acid and the absence or presence of a mucous metaplasia. Puromycin is an antibiotic known to interfere with the translation mechanism.

Materials and methods. 29 male rabbits (average weight, 1 kg) had the inner surface of their right ear auricles painted twice weekly with 1% 7,12-dimethylbenzanthracene (DMBA) in equal parts of lanolin and mineral oil. After 6 weeks, all the rabbits had developed 6–7 keratoacanthomas per ear (0.5–1 cm in diameter). The animals were divided into several groups. The protocol of the materials and experimental methods employed in the study are summarized in the Table. The corresponding left ears of all the rabbits served as controls.

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All biopsied tissues, whether from experimental or control tissues, were incubated in 4% glutaraldehyde in phosphate buffer for 1.5 h followed by fixation in 2% osmium tetroxide buffered to pH 7.4. The tissues were dehydrated in graded strengths of ethanol and embedded in Epon 812. 1 μm thick and ultrathin sections were cut on a Reichert ultramicrotome. The ultrathin sections were stained with uranyl acetate followed by lead citrate, and examined in an RCA type EMU 2E electron microscope.

Results. Gross morphology. Studies previously carried out in this laboratory have demonstrated that the usual dry, keratotic keratoacanthoma will exude a viscous and copious exudate when subjected to 3 to 5 daily topical applications of 0.5 g of 3% vitamin A acid. In the present study, when 1 or 2 applications of puromycin were applied either prior to or after vitamin A acid, there was a visible exudate present at 72 h on the surface of the keratoacanthomas. When puromycin was applied concurrently with vitamin A acid, there was never any visible exudate on the tumors.

Microscopic results. The ultrastructure of normal rabbit hair follicle, the keratoacanthoma, and the vitamin A acid-treated keratoacanthoma has been previously reported[3–5]. When tumor keratinocytes are subjected to 1 or 2 doses of puromycin, glycogen is present in the cytoplasm. When the epithelial cells are subjected to 1 or 2 doses of puromycin followed by 1 to 4 doses of vitamin A acid, there is an increase in the Golgi and endoplasmic reticulum at 72 h. The daily applications of puromycin concurrently with 5 daily applications of vitamin A acid produces only sparse amounts of Golgi and rough endoplasmic reticulum. When 2 or 3 doses of vitamin A acid are applied prior to multiple doses of puromycin, the tumor keratinocytes develop both dilated cisternae of the rough-surfaced endoplasmic reticulum (Figure 1) and the Golgi reticulum.

Examination of the control tissues that received the same dosages and schedule of drugs as the tumorous tissues revealed similar morphologic results. The untreated keratinocyte possesses both a sparse rough-surfaced endoplasmic reticulum and Golgi reticulum (Figure 2).

Discussion. In several previous studies, the topical application of vitamin A acid to dry, keratotic keratoacanthomas has resulted in a metaplasia in the keratinocytes with the production of mucigen droplets in the cytoplasm and a viscous, copious mucus at the macroscopic level[4,5].

In the present study, puromycin applied prior to or after vitamin A acid applications did not prevent mucus production or secretion. When puromycin is given concurrently with vitamin A acid for 5 days, no mucus exudate is present. The effect of puromycin is observed when it is continually applied.

It appears that continual applications of puromycin prevents the Golgi reticulum from maturing to the developmental stage that is observed when only vitamin A acid is applied. FRIEDMAN and CARDELL[9], in studies on

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A protocol of the experimental materials and methods used in the study

Experiment	No. of tumors	Topical application of puromycin or vitamin A acid	Time interval between drugs	Time of biopsy
1	9	1 dose of puromycin[a]	2 h later 1 dose of vitamin A acid[b]	In this experiment and all succeeding experiments, biopsies were taken at 4, 6 and 24 h except where noted
2	11	2 doses of puromycin 2 h apart	2 h later 1 dose of vitamin A acid followed 24 h later by a 2nd dose of vitamin A acid	
3	38	2 doses of puromycin 2 h apart	2 h later 1 dose of vitamin A acid. 14/38 tumors received 2 further doses of vitamin A acid 24 h apart. 14/38 tumors received a 4th dose of vitamin A acid 24 h later and 10/38 tumors received a 5th dose of vitamin A 24 h later	
4	15	1 dose of puromycin given concurrently with 1 dose of vitamin A acid daily for 5 days		3 tumors biopsied daily 24 h apart for 5 days
5	8	1 dose of puromycin		
6	9	2 doses of puromycin		
7	27	2 doses of vitamin A acid 24 h apart	14/27 tumors received 1 dose of puromycin 2 h later. 13/27 tumors received a 2nd dose of puromycin 2 h later	
8	18	3 doses of vitamin A acid 24 h apart	9/18 tumors received 1 dose of puromycin 2 h later. 9/18 tumors received a 2nd dose of puromycin 2 h later	
9	9	5 doses of vitamin A acid 24 h apart	All 9 tumors received 2 doses of puromycin 2 h apart 2 h after 5th application of vitamin A	

[a] Puromycin, 500 μg in 0.1 ml of acetone. [b] 0.5 g of 3% vitamin A acid in lanolin and mineral oil in equal parts.

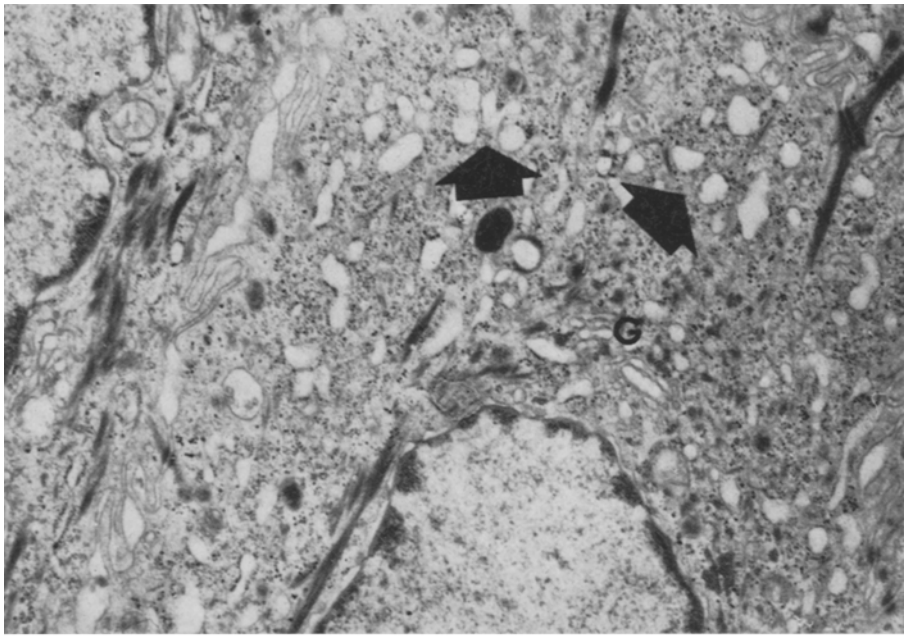

Fig. 1. Micrograph depicts dilated cisternae of the rough surfaced endoplasmic reticulum (arrows). Golgi apparatus (G) is present. ×12,000.

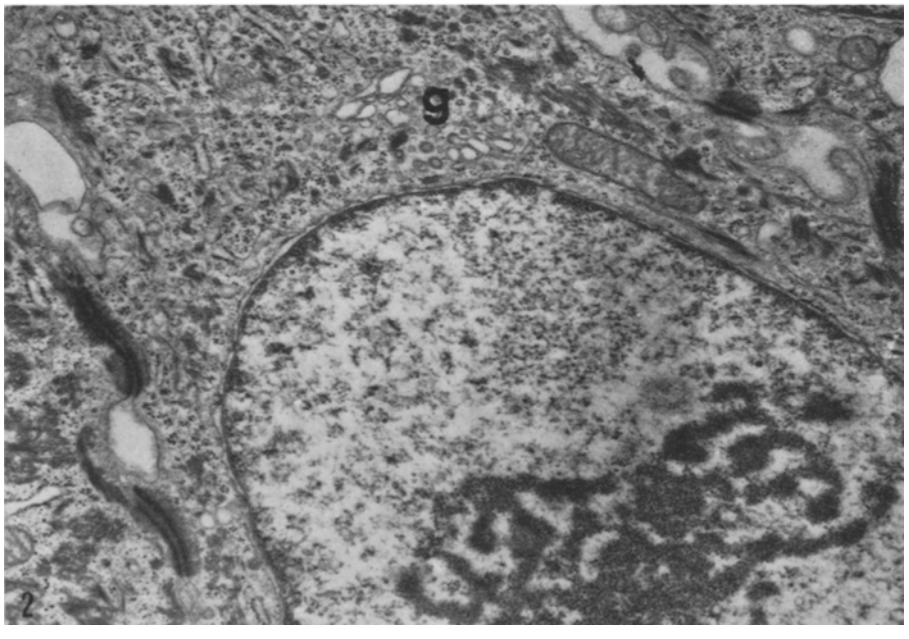

Fig. 2. Micrograph depicts Golgi reticulum (g) in untreated keratinocyte. ×12,000.

the effect of puromycin on rat intestinal epithelial cells during fat absorption, noted that puromycin treatment causes a deficiency of Golgi membranes. Present evidence indicates that the Golgi saccule is the site where carbohydrate is synthesized and added to protein, the latter synthesized on the ribosomes of the rough endoplasmic reticulum[10–12]. NEUTRA and LEBLOND[13,14] have reported that the Golgi saccule eventually becomes a mucigen droplet. The loss of all mucigen droplets with puromycin may be due to the fact that puromycin inhibits the incorporation of sugar and sulfate into mucopolysaccharide[15,16].

Zusammenfassung. Das mikroskopische Bild des schnell wachsenden Keratoakanthoms beim Kaninchen wird durch Vitamin A und Puromycin wesentlich beeinflusst und verändert.

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