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22 October 1982

Digoxin-Inactivating Bacteria: Identification in Human Gut Flora

Abstract. *Digoxin, the most widely used cardiac glycoside, undergoes significant metabolic conversion in many patients to cardioinactive metabolites in which the lactone ring is reduced. This appears to occur within the gastrointestinal tract. An attempt was made to isolate and identify the organisms capable of reducing digoxin from stool cultures obtained from human volunteers. Of hundreds of isolates studied, only* Eubacterium lentum, *a common anaerobe of the human colonic flora, converted digoxin to reduced derivatives. Such organisms were also isolated in high concentrations from the stools of individuals who did not excrete these metabolites when given digoxin in vivo. When the growth of* E. lentum *was stimulated by arginine, inactivation of digoxin was inhibited. Neither the presence of these organisms alone nor their concentration within the gut flora appeared to determine whether digoxin would be inactivated by this pathway in vivo.*

The cardiac glycoside digoxin (Fig. 1) is the most widely used drug in the treatment of heart disease and the seventh most commonly prescribed medication in the United States (*1*). Although earlier studies suggested that digoxin escaped metabolic degradation and was excreted from the body largely unaltered, recent work indicates that the drug is metabolized in a substantial minority of patients (*2, 3*). Approximately one in ten individuals taking digoxin excretes large amounts of reduced metabolites, such as dihydrodigoxin (Fig. 1), in which the lactone ring on the molecule is saturated (*3, 4*). Such digoxin reduction products (or DRP), which bind poorly to the cardiac receptor site (membrane-associated Na^+,K^+–dependent adenosinetriphosphatase), are only minimally concentrated by cardiac tissue, undergo rapid excretion, and possess much less cardiac activity than digoxin (*5–7*). Patients who make massive amounts of DRP may have strikingly increased requirements for the parent drug (*4, 8*). It has not been determined why most patients treated with digoxin consistently make no reduced metabolites or only trivial amounts (so-called DRP nonexcretors), whereas others consistently form moderate to marked quantities of DRP (excretors) (*3*).

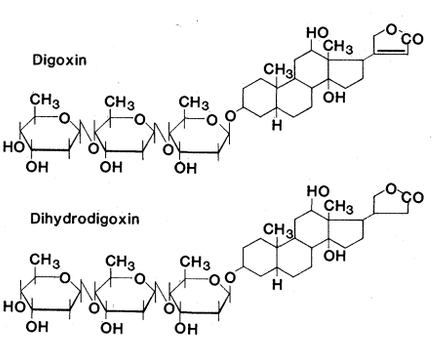

Fig. 1. The single double bond in the lactone ring of digoxin (above) is reduced in dihydrodigoxin (below).

We recently demonstrated that DRP were formed in the gastrointestinal tract of excretor subjects apparently exclusively as the result of the action of enteric bacteria (*7*). The organisms responsible for the conversion of digoxin to DRP have not previously been identified. We report here the isolation and identification of anaerobic organisms present in the human gut flora capable of reducing digoxin, as well as experiments designed to test the hypothesis that variation in the concentration of such organisms accounts for differences in the metabolic behavior of excretor and nonexcretor subjects.

Fresh stool samples from two human volunteers who were known to be heavy DRP excretors were cultured anaerobically in chopped meat glucose broth (Scott Laboratories) containing digoxin

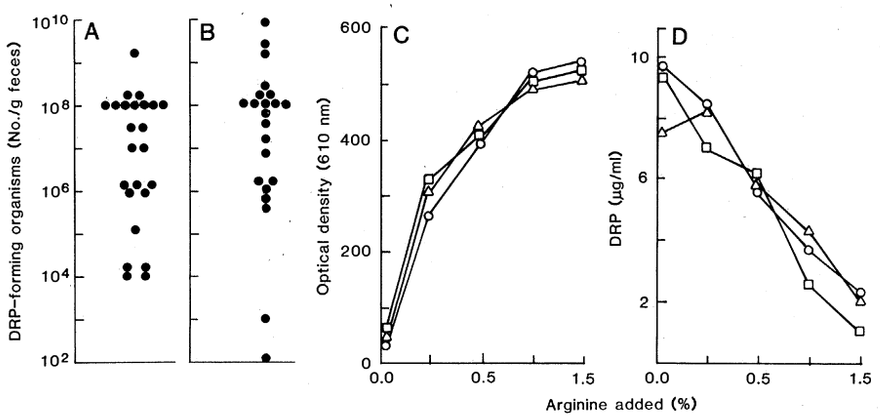

Fig. 2. (A and B) Concentration of DRP-forming organisms per gram of feces in (A) 24 of the 25 excretors and (B) 22 of 47 nonexcretors whose stools produced DRP in vitro. Concentrations were calculated from the maximum dilution of stool positive for DRP, based on wet weight of feces. (C and D) Effects of addition of arginine to pure cultures of three DRP-forming *Eubacterium lentum* strains on (C) growth and (D) DRP production. Arginine base was added in increasing concentrations to previously reduced brain heart infusion broth (*9*) containing digoxin, 10 μg/ml. Single strains of *E. lentum* were incubated for 48 hours and growth was measured as increments in optical density compared to uninoculated controls. DRP in supernatants was determined by radioimmunoassay. Two of the strains were stool isolates from an excretor and a nonexcretor subject. The third was a clinical isolate from a patient with an infection.

at a concentration of 10 μg/ml (CMGD). After 5 to 7 days of incubation at 37°C, the concentrations of digoxin and DRP in the supernatants were measured by separate radioimmunoassays (*3, 6*). The supernatants of the stool cultures from both subjects converted to DRP 100 percent of the digoxin originally present. Sheep blood agar plates were then inoculated with the DRP-positive cultures and incubated at 37°C in Gas Pak jars (Baltimore Biological Laboratories) for 48 to 96 hours. Isolated colonies were selected randomly and cultured in CMGD, and the supernatants assayed for DRP formation. Of more than 400 colonies selected, only two were found to produce DRP. Both were identified as *Eubacterium lentum* by means of standard methods, including biochemical fermentations and gas-liquid chromatography (*9*). *Eubacterium lentum* is a non-sporeforming anaerobic Gram-positive rod identified by its lack of reactivity in standard tests; it is asaccharolytic and nonproteolytic and produces little or no volatile fatty acids (*9*). It is of interest, in view of the digoxin reducing ability of the organism, that strains of *E. lentum* have been shown to transform a variety of other steroid compounds, including bile salts and progesterone (without utilizing them as substrates) (*10*).

We then cultured 150 stock strains of anaerobes and aerobes (*11*) representative of the normal human gut flora (*12, 13*) in CMGD broth and tested for DRP production. The only organisms found to elaborate DRP were strains of *E. lentum*. Of 13 *Eubacterium* species tested, only *E. lentum* formed DRP; of 28 strains of *E. lentum* studied, 18 made DRP, including the type strain (American Type Culture Collection No. 25559).

We then sought to determine whether excretor subjects could be distinguished from nonexcretors by the presence or absence of DRP-producing *E. lentum* in the gut flora or by the concentration in feces of such organisms. Stool specimens were obtained from 72 healthy normal volunteers. They were then given 0.2 mg of digoxin by mouth and their excretor status was determined by radioimmunoassays for digoxin and DRP on urine collected for 48 hours (*3*). Twenty-five subjects were DRP excretors and 47 were nonexcretors. Serial tenfold dilutions of the fresh stool specimens were made in CMGD broth under anaerobic conditions (*9*), and after incubation the diluted specimens were assayed for DRP. One or more dilutions of stool from 24 of the 25 excretor subjects made DRP; the maximum dilution in which DRP was produced is shown in Fig. 2A. No DRP was formed at any dilution by the stool cultures from 25 of the 47 nonexcretors. Of interest, however, and in contrast to the results of an earlier study in which we used a different culture technique with a limited number of subjects (*7*), stool cultures from the remaining 22 nonexcretors produced DRP. Furthermore, the maximum positive dilutions were similar to those found in excretors (Fig. 2B). Using the methods described above, we then isolated five organisms that produced DRP from the stools of two subjects who were repeatedly shown to excrete no detectable DRP when given digoxin on multiple occasions. Each of these five organisms was identified as *E. lentum*.

Our data indicate that a rather limited group of organisms, certain strains of the species *E. lentum*, is responsible for the conversion of digoxin to DRP in the gastrointestinal tract of excretor subjects. This activity is demonstrable in high dilutions of stool, consistent with reports that this species is often present in high concentrations in the normal gut flora (*13*). The mere presence of these organisms alone cannot account for the tendency of some individuals to reduce digoxin, since almost half of the nonexcretors that we examined also harbor DRP-forming bacteria at similarly high concentrations. Prolonged exposure to digoxin during long-term therapy does not induce the formation of DRP in nonexcretors (*3*). What then, is responsible for the difference in the metabolic activity of the gastrointestinal flora of excretor as opposed to nonexcretor subjects?

As mentioned earlier, *E. lentum* lacks the ability to metabolize various substrates utilized by other enteric bacteria. The only substrate known to stimulate the growth of this organism is the amino acid arginine (*14*). We therefore studied the effect of growth enhancement with arginine on DRP formation by this species. Representative experiments are shown in Fig. 2, C and D. As increasing concentrations of arginine produced corresponding increments in growth, DRP production declined. Similar results were obtained with *E. lentum* strains obtained from excretors and nonexcretors, as well as subcultures of whole stool from excretor subjects.

Thus, the inactivation of digoxin by way of the reduction of its lactone ring appears to be mediated in the human gastrointestinal tract by a subset of a single species of bacteria, *Eubacterium lentum*, that is a common constituent of the normal gut flora. Not all persons who harbor such bacteria at high fecal concentrations, however, will excrete reduced metabolites during digoxin therapy. Inhibition of the expression of this metabolic reaction presumably occurs in vivo in these individuals. Conceivably, high concentrations of arginine at the site in the lower gastrointestinal tract at which *E. lentum* are exposed to digoxin might account for such inhibition. However, many other factors may influence enzymatic reactions mediated by intestinal microorganisms. Further studies are required to determine why the gut flora of some but not all patients inactivate digoxin; these should lead to greater

understanding of the mechanisms controlling the microecology and metabolic activity of the human gut flora, as well as more consistently effective drug therapy.

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Visual Sustained Attention: Image Degradation Produces Rapid Sensitivity Decrement Over Time

Abstract. *Perceptual sensitivity to a visual target presented in a random continuous sequence of targets and nontargets decreased rapidly over time when stimuli were highly degraded visually but not when moderately degraded or undegraded. Large declines in sensitivity, independent of changes in response criterion, were found after only 5 minutes of observation. These rapid decrements of sensitivity to degraded targets seem to result from demands on the limited capacity of visual attention.*

The capacity to sustain attention to visual targets typically deteriorates over a period of continuous observation. The decrement in target detection rate can result from a loss in perceptual sensitivity, from changes in response or decision criteria, or from both (*1, 2*). Sensitivity declines have been linked to the combination of a high stimulus processing rate and a target that requires memory for successive stimuli (*1, 3*). Such sensitivity decrements are generally small and occur only after about 30 to 45 minutes (*1–3*). However, Nuechterlein has recently developed a task requiring detection of degraded visual targets without memory for successive stimuli that appears to elicit sensitivity decrements within time periods as short as 5 to 10 minutes (*4, 5*) when single-response data (yes responses) are evaluated. The task is an adaptation of the continuous performance test (CPT) commonly used to assess clinical populations (*6*); image degradation is used to burden early stimulus encoding and analysis during information processing.

We now show through derivation of receiver operating characteristic (ROC) curves that rapid decrements in perceptual sensitivity over time occur as a function of degree of image degradation. Repeated sessions of observation did not abolish the sensitivity decrement. Sensitivity decrements under such conditions occurred more rapidly than those reported for other sustained attention tasks (*1, 7*).

Volunteers, 21 males and 21 females, aged 17 to 23 years and with normal (20/20) or fully corrected vision, participated. Each subject sat 1 m from a rear projection screen on which single digits (0 to 9) were presented for 40 msec every 1 second. Subjects indicated their confidence each time that the digit 0 was detected by depressing one of three response keys on a terminal, the three keys being labeled "sure," "not so sure," and "unsure." No response was required for nontarget digits. Targets were presented irregularly with a probability of .25. Target and nontarget digits were presented in a pseudorandom sequence with the restrictions that identical digits never follow one another and that targets be preceded by each nontarget digit an equal number of times. A total of 486 stimulus trials (120 targets and 366 nontargets) were presented over an observation period lasting just over 8 minutes. Although the stimuli were presented continuously, trials were divided into three 2.7-minute blocks of 162 trials for analysis.

Stimuli were presented with a Kodak Carousel model E-2 slide projector (focal length 6 inches) fitted with an Ilex No. 4 Synchro Electronic shutter and Ilex Speedcomputer. The digit stimuli subtended approximately 3° of visual angle vertically and 2° horizontally. Illumination from the screen was 159 lux with the projector lamp off and 191 lux with it on. A visual mask, consisting of a transparency containing typed plus (+) characters, was mounted on the back of the rear projection screen to decrease figure-ground contrast and visual persistence. Stimuli were degraded at three levels—low, moderate, and high—by decreasing the object (slide-to-lens) distance for a fixed slide-to-screen distance (by blurring or defocusing the image). The power of a correcting lens, P_c, required to restore the image to the undegraded (focused) level at the same screen distance indexed the degree of image degradation (*8*). Phenomenally, the digits appeared almost focused at the low level, blurred at the moderate level, and highly blurred at the high level of image degradation.

Subjects were assigned randomly to one of the three image degradation conditions (with the restriction that each group contain an equal number of males and females). Subjects were given a minimum of 200 trials of practice before performing the task. During the practice period, targets and nontargets were pre-