of the expression of IL-2 receptors was not mediated by IFN-γ (data not shown).

Dex and anti-Tac reduced the number of receptor-bearing cells in cultures supplemented with Con A alone. T cells treated with PMA and then cultured with Con A were more resistant to the inhibitory effect of Dex on the expression of IL-2 receptors. This inhibitory effect on cells activated with lectin could be reversed by exogenous IL-2. IL-2 also increased the number of receptor-bearing cells in cultures supplemented with Dex and induced with PMA and Con A. The combined effect of Dex (inhibition of IL-2 synthesis) and anti-Tac (blockage of IL-2 receptors) was synergistic. The expression of receptors induced with Con A alone or in combination with PMA was almost completely abrogated by anti-Tac in combination with Dex. Exogenous IL-2 had only a moderate effect in preventing this inhibition.

Synthesis of IFN-γ was modulated by IL-2 in a manner resembling the effect of IL-2 on its receptors. IL-2 served as an amplifying signal by augmenting IFN-γ synthesis. T cells cultured in complete medium produced low amounts of IFN-γ in culture, and exogenous IL-2 moderately increased their production of IFN-γ. This low degree of synthesis was increased by PMA and Con A. Maximum synthesis of IFN-γ was observed in cultures induced with Con A and PMA to which IL-2 was added. Maximum inhibition was achieved by the combined effects of Dex and anti-Tac. Dex inhibited synthesis of IFN-γ by T cells induced with Con A alone or in combination with PMA. This inhibition was readily overcome by IL-2 in cultures supplemented with Con A and was partially reversed in cultures containing PMA and Con A. Anti-Tac inhibited IFN-γ synthesis more effectively in T cells activated with Con A than in T cells cultured with PMA and Con A. Once the expression of IL-2 receptors was blocked by anti-Tac, IL-2 was not effective in reversing the inhibitory effect.

In conclusion, IL-2 has an important role in augmenting the expression of IL-2 receptors and in the synthesis of IFN-γ activated by T cells. In cultures of T cells whose supply of IL-2 has been cut off by Dex and anti-Tac, the number of cells displaying receptors and the density of receptors are decreased, and synthesis of IFN-γ ceases. This regulatory effect of IL-2 precedes T cell proliferation. The observation that Dex, an effective immunosuppressant drug, interferes with IFN-γ synthesis may be of clinical significance. Patients treated with glucocorticoids such as Dex risk infection partly because Dex inhibits the bacteriocidal effect of macrophages. Since IFN-γ is an activator of macrophages, these patients could be protected from infections by administration of IFN-γ.

Note added in proof: A similar observation that IL-2 was required for the optimal expression of IL-2 receptors on activated T cells has been made (*8*).

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9. We thank K. Welte and K. A. Smith for purified IL-2, T. Uchiyama for anti-Tac, and T.-W. Chang for help with the determination of IFN-γ. This work was supported by NIH grant RO 1 CA 33653-01A1.

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Intestinal Uptake and Metabolism of Auranofin, A New Oral Gold-Based Antiarthritis Drug

Abstract. *Auranofin, 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-*D*-glucopyranosato-*S*-(triethylphosphine)-gold(I), an experimental antiarthritis pharmaceutical, metabolized in contact with hamster or rat gut wall to yield the deacetylated form of the drug. This product, 1-thio-*β*-*D*-glucopyranosato-*S*-(triethylphosphine)gold(I), passed through hamster or rat intestinal wall in an everted gut experiment. The metabolite was separated by high-performance liquid chromatography and characterized by retention time, chemical reactivity to yield a known product, and comparison to a synthetic sample of the metabolite.*

There is considerable evidence that auranofin, currently awaiting approval by the Food and Drug Administration for use in the United States (*1*), is effective in inducing remission of rheumatoid arthritis (*2*). However, little is known about the site of uptake of the drug, which is given orally, or the nature of the compounds circulating in patients after its administration. We used the everted sac model of intestinal absorption (*3*) to study uptake of auranofin. When everted intestinal sacs were incubated with a solution of auranofin for 2 hours, the gold concentration inside the sacs was about 20 percent of that in the incubation medium. Exposure to the intestinal tissue resulted in metabolism of auranofin. The product that accumulated inside the sac was the deacetylated form of the drug.

The everted sac preparation involves dissecting the intestine into segments, turning each segment inside-out, and tying it into a sac (*3*). The absorptive (mucosal) surface is thus exposed to the incubation medium, and the material that accumulates inside the sac has passed through the intestinal wall. For our experiments we used Syrian golden hamsters (150 g) and Sprague-Dawley rats (strain F344; 150 to 200 g). The intestine removed from an anesthetized animal was divided into two jejunum and two ileum segments. Segments were everted over polyethylene tubing, filled with Krebs-Ringer solution with bicarbonate buffer and 1 mg of glucose per milliliter, and tied into sacs. Sacs were incubated in this medium with auranofin for 2 hours at 37°C in a shaker moving at 1.33 Hz. The atmosphere was flushed continuously with 95 percent O_2 and 5 percent CO_2.

Both the incubation medium and the material collected from inside the sacs were assayed for glucose with Sigma kit 510 and for gold with inductively coupled plasma atomic emission spectroscopy (*4*). Since only those preparations in which glucose accumulates in the sacs against the concentration gradient maintain functional tissue, only those were analyzed further (typically nine of ten preparations). In these experiments the concentration of gold in the incubation medium ranged from 40 to 80 ppm. After 2 hours the concentration of gold in the sacs ranged from 15 to 30 percent of

that in the incubation medium, showing that gold does pass through the intestinal wall in this model system. There was no evidence for a difference in uptake between jejunum and ileum segments.

We used high-performance liquid chromatography (HPLC) to characterize the gold-containing material that accumulated in the intestinal sacs (Fig. 1) (*5*). The chromatogram in Fig. 1A represents a sample from an everted sac that had been incubated with a solution containing an excess of solid auranofin. Under these conditions the concentration of gold inside the sac reached approximately 50 ppm, and the material represented by the chromatographic peaks accounted for at least 90 percent of the total gold. The point on the chromatogram marked with an arrow is at the retention time observed for auranofin, and the chromatogram indicates less than 0.2 ppm auranofin inside the sac. Therefore, any gold species inside the sac must be an auranofin metabolite rather than auranofin itself. There is a major ultraviolet peak at 4.5 minutes (on the trailing edge of the off-scale peak for nonretained materials) and there are two minor peaks around 5.5 minutes. Since the peak at 4.5 minutes is not found in chromatograms of control samples not exposed to auranofin, the peak appears to represent a metabolite of the drug.

The chromatogram of an identical sample from inside the sac mixed with a solution of 2,3,4,6-*O*-acetyl-1-thio-β-D-glucopyranose (tetraacetylthioglucose) is shown in Fig. 1B. The major peak at 4.5 minutes and the minor ones at 5.5 minutes are gone and a peak is now found at the retention time for auranofin. The peak at 5.0 minutes in Fig. 1B is from the excess tetraacetylthioglucose. Reformation of auranofin by the addition of tetraacetylthioglucose requires that the triethylphosphinegold portion of the molecule remain intact. The formation of multiple metabolites, all of which appear to reform auranofin, suggests that the metabolism involves modification of the tetraacetylthioglucose moiety of auranofin rather than simple Au-S cleavage.

One possible modification is complete deacetylation. The deacetylated form of auranofin, 1-thio-β-D-glucopyranosato-(triethylphosphine)gold(I), was synthesized from chloro(triethylphosphine)-gold(I) and the thiosugar. The chromatogram of that material is shown in Fig. 1C. The retention time, 4.5 minutes, is identical to that of the gut metabolite. Also, combination of the gut metabolite with the synthetic product gave a chromatogram with a single peak at 4.5 minutes, unchanged in shape from that of either of the individual components (*6*). Furthermore, addition of tetraacetylthioglucose to deacetylated auranofin resulted in conversion to auranofin (Fig. 1D).

On the basis of retention time and reaction with tetraacetylthioglucose to reform auranofin, the major gold species in the intestinal sac after incubation with auranofin is 1-thio-β-D-glucopyranosato-(triethylphosphine)gold(I). This conversion of auranofin to the deacytylated derivative is clearly dependent on the intestinal tissue. Auranofin dissolved in Krebs-Ringer bicarbonate buffer solution (*p*H 7.4) was stable during incubation for 1 week at 37°C (*7*). Experiments with rats and hamsters gave similar results, indicating that the uptake and metabolism are not species-specific.

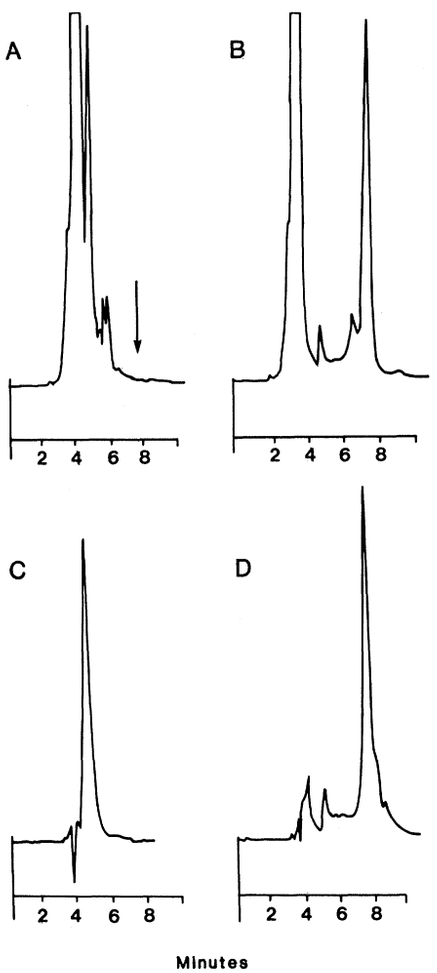

Fig. 1. High-performance liquid chromatograms of auranofin and metabolites obtained with a reversed-phase C_{18} column and methanol-water solvent (*5*). (A) Chromatogram of the solution inside the everted gut sac incubated with Krebs-Ringer bicarbonate buffer and excess auranofin. The off-scale peak resulted from materials not retarded by the column; the sharp peak immediately after it (at 4.5 minutes) resulted from the auranofin metabolite. (B) Same sample as represented in (A), but mixed with tetraacetylthioglucose before injection. The peak at 7.5 minutes is characteristic for auranofin. (C) Sample of synthetically prepared thioglucose(triethylphosphine)gold(I) with a retention time identical to that of the metabolite represented in (A). (D) Sample of the material represented in (C), mixed with tetraacetylthioglucose prior to injection. Conversion of the synthetic material to auranofin is shown.

Little information is available on the site of uptake after administration of auranofin or on the gold species taken up. One study (*8*) suggested loose, reversible adsorption of auranofin to the enteric cell surface rather than true transmucosal absorption. Another study, which suggested uptake by the stomach (*9*), was done with chloro-(triethylphosphine)gold(I) and not auranofin. The results of a third study (*10*) involving radioactive labels (^{35}S and ^{32}P) were interpreted as showing that a large amount of Au-S and Au-P bond disruption may have occurred in the intestine before absorption. While not contradicting any of these findings, our experiments show greatly different results. First, gold-containing species formed from auranofin do pass through the intestinal wall, and second, neither Au-S nor Au-P bond disruption takes place in the initial metabolic steps—rather, the tetraacetylated sugar is stripped of the four acetyl groups.

These observations have implications for studies of auranofin in vivo and in vitro. Such studies have dealt with protein binding, chemotaxis, hydrolase activity, phagocytosis, immune response, DNA synthesis, and other topics (*11*). Since it appears that little or no auranofin passes through the intestinal wall and that the deacetylated form of the drug does, it might be advisable to conduct these studies with the latter material. Certainly it is important to determine the form of gold circulating in patients after auranofin has been administered.

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4. For high concentrations and clear samples, the conventional solution nebulization sample introduction was used; for solutions with suspended particles or small volumes, the electrothermal vaporization method for sample introduction was used [K. C. Ng and J. A. Caruso, *Anal. Chem. Acta* **143**, 209 (1982)].
5. The HPLC system consisted of a BAS high-

performance liquid chromatograph with a Beckman ultraviolet detector operated at 214 nm with a zinc lamp. Samples were introduced with a 20-μl Rheodyne loop or a 50-μl Hamilton syringe. The mobile phase consisted of a 65:35 solution of methanol and 0.25 percent (weight to volume) aqueous ammonium dihydrogen phosphate buffer with a flow rate of 0.8 ml/min. The column (length, 25 cm; inner diameter, 4.5 mm) was an Apex model commercially packed with octadecylsilane reversed-phase material (particle size, 5 μm; Jones Chromatography). Experiments were performed at ambient temperature. A calibration curve was determined by plotting the auranofin peak height versus the gold concentration (in parts per million) of stock auranofin solutions. All solutions were filtered through 0.45- or 0.22-μm membrane filters before use.

6. Although the chromatographic peak at 4.5 minutes could have resulted from multiple components with similar retention times, the fact that the shape of the peak did not change when the synthetic single material 1-thio-β-D-glucopyranosato(triethylphosphine)gold(I) was added indicates that this material was the only component contributing to the peak.
7. Auranofin incubated in 0.01*M* HCl for 7 hours is largely unchanged; thus the bulk of auranofin ingested may be expected to reach the intestine intact [I. C. P. Smith, A. Joyce, H. Jarrell, B. M. Sutton, D. T. Hill, in *Bioinorganic Chemistry of Gold Coordination Compounds*, B. M. Sutton and R. G. Franz, Eds. (Smith Kline & French Laboratories, Philadelphia, 1983), pp. 47–57]. Apparently, contact with the mucosal cell surface initiates the deacetylation reactions since auranofin in the incubation medium outside the sac shows significant deacetylation as well.
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Single Channel Studies on Inactivation of Calcium Currents

Abstract. *Inactivation of calcium channels has been attributed to a direct reaction of calcium ions entering the cell with the calcium channel. For a single channel this hypothesis predicts a correlation between the amount of calcium entering during an opening or a burst of openings and the subsequent closed times. No such correlation was found, and the possibility that, upon entry, calcium ions produce inactivation is excluded.*

Activation of calcium channels in excitable membranes is voltage-dependent (*1*, *2*), but inactivation has been described as voltage-dependent or current-dependent or both (*3*, *4*). The current-dependent hypothesis attributes inactivation to a direct reaction between calcium ions that have entered the channel and the channel itself (*4*, *5*). The opening of a single calcium channel provides an instantaneous and quantitative calcium injection, and the effect on inactivation can be investigated by observing subsequent single channel behavior.

Our experiments were performed with neuronal cell bodies in the right parietal ganglion of *Helix pomatia* with the use of the scheme shown in Fig. 1A, in which whole-cell and gigaseal ($>10^9\Omega$) patch clamp currents were recorded simultaneously (*6*). A sodium-free solution containing 40 m*M* $CaCl_2$, 5 m*M* $MgCl_2$, 35 m*M* tetraethylammonium chloride, 5m*M* 4-aminopyridine, and 20 m*M* tris (*p*H 7.4) was used in the bath and patch pipettes. For measurements of barium current, barium ion was substituted isosmotically for calcium ion.

Unitary currents (Fig. 1B) were caused by the flow of calcium ions from the pipette into the cell. The rise and fall of averaged single-channel and whole-cell currents were similar. The time course of activation and inactivation increased with potential over the range of −25 to +5 mV. Changing the holding potential from −50 to −90 mV had no effect on single channel behavior during steps to these same potentials. Barium currents have also been studied and compared to calcium currents (*6*). The averaged single-channel and whole-cell barium currents also overlaid each other when scaled. The results showed that the average behavior of a single calcium or barium channel in a patch of membrane was the same as the behavior averaged from all the activated calcium or barium channels in the cell.

Some possible causes of inactivation can be excluded. The absence of outward unitary currents established that inactivation was a property of single calcium channels. Mean open times and amplitudes of openings were compared between the rising and falling phases (Fig. 2). These were unchanged as were the open time distributions, which were fitted by the same single-exponential function during the two phases. Therefore, neither a change in unitary conductance nor in open state caused inactivation. The closed times were distributed in at least a double-exponential manner (*7*), and the average values of the closed times during the first 50 to 75 msec which encompassed the peak of the current were compared with the average values during the last 200 msec, when the current was inactivating and had become almost steady. The average value of the closed time increased approximately threefold between these times, which compares well with the reduction of the frequency of opening in the averaged currents (Fig. 1B).

We deduced that if inactivation were due to a cytoplasmic process produced

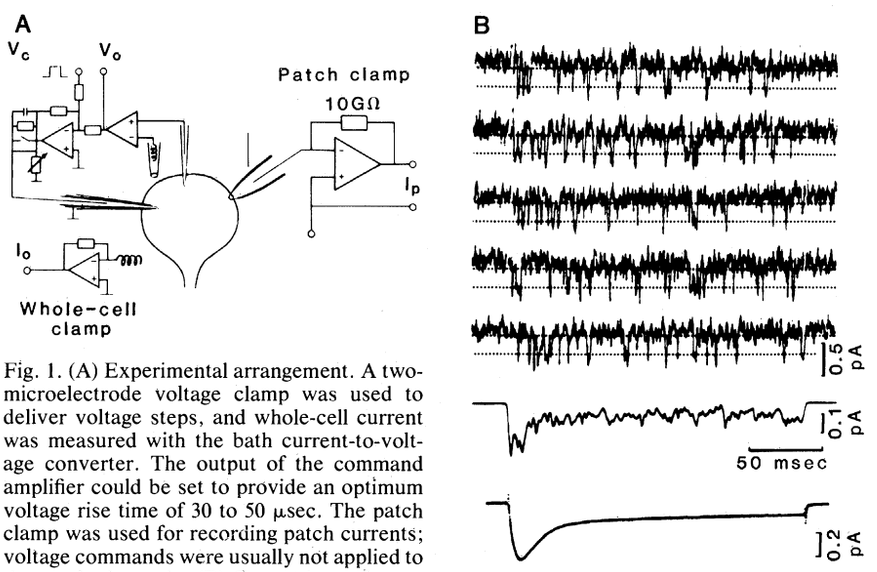

Fig. 1. (A) Experimental arrangement. A two-microelectrode voltage clamp was used to deliver voltage steps, and whole-cell current was measured with the bath current-to-voltage converter. The output of the command amplifier could be set to provide an optimum voltage rise time of 30 to 50 μsec. The patch clamp was used for recording patch currents; voltage commands were usually not applied to the patch electrode. (B) Comparison of averaged single-channel and whole-cell currents. Five samples of single-channel events are shown. Immediately below is the averaged current obtained from 42 single-channel records, in which one failure occurred. The bottom record shows a whole-cell current that was recorded simultaneously, showing a similar inactivation time course. Holding potential (V_H) was −50 mV; test potential was to a membrane potential (V_M) of 0 mV; pulse duration was 200 msec; intervals between pulses were 10 seconds. Temperature was 29°C to increase rates of activation and inactivation. The effects were also present at 20°C. Bandwidth was 1.0 kHz and sampling rate was 5 kHz. Threshold for openings was about three times the root mean square of the background noise and for closing about 1.5 times the root mean square of the noise.