Hypothesis

New uses for old drugs. Auranofin, a clinically established antiarthritic metallodrug, exhibits potent antimalarial effects *in vitro*: Mechanistic and pharmacological implications

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Abstract **The clinically established gold-based antiarthritic drug auranofin (AF) manifests a pronounced reactivity toward thiol and selenol groups of proteins. In particular, AF behaves as a potent inhibitor of mammalian thioredoxin reductases causing severe intracellular oxidative stress. Given the high sensitivity of *Plasmodium falciparum* to oxidative stress, we thought that auranofin might act as an effective antimalarial agent. Thus, we report here new experimental results showing that auranofin and a few related gold complexes strongly inhibit *P. falciparum* growth *in vitro*. The observed antiplasmodial effects probably arise from direct inhibition of *P. falciparum* thioredoxin reductase. The above findings and the safe toxicity profile of auranofin warrant rapid evaluation of AF for malaria treatment in animal models.**
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1. Introduction

Since the 1930s, a number of injectable gold(I) thiolates drugs, in particular myochrysine, sanochrysine, allochrysine, and solganol [1,2]. have been widely employed in the clinics for the treatment of rheumatoid arthritis (the so called *Chrysotherapy*). Later on, in 1979, a new antiarthritic gold(I) phosphine compound, ((2,3,4,6-tetra-*O*-acetyl-1-(thio-kS)-β-D-glucopyranosato)(triethylphosphine) gold(I)), auranofin (AF), (Fig. 1) was introduced in the clinics, with the significant advantage of an oral administration [3]. Today, *chrysotherapy* still represents an effective option for the treatment of severe forms of rheumatoid arthritis though it is being progressively replaced by new and more sophisticated therapeutic approaches.

In spite of their long and established clinical use, the mechanism of action of antiarthritic gold drugs is still largely controversial. However, recent studies point out that antiarthritic gold drugs directly interfere with the functioning of the immune system, at various levels [4,5]. In addition, a number of biochemical studies revealed that gold drugs behave as potent "*thiol reactive species*" and act as effective inhibitors of a number of proteases (mostly cysteine proteases) [6,7], involved in the progression of rheumatoid arthritis (RA). It also emerged that auranofin and some related gold drugs are strong inhibitors of mammalian thioredoxin reductases (TrxR), both in the cytosol and in the mitochondria [8–10]. Notably, thioredoxin reductase inhibition is associated with important modifications of the intracellular redox balance and, in particular, with induction of severe oxidative stress and relevant cytotoxic effects *in vitro* [11,12].

2. Auranofin: a possible antimalarial agent?

This latter feature of AF, i.e. its ability to act as a strong inhibitor of thioredoxin reductase (in the nM range), suggests that AF might display additional pharmacological uses beyond RA therapy. Indeed, thioredoxin reductases are emerging in the last years as an important class of likely drug targets for a variety of diseases [13]. Various research groups formerly analyzed the possible use of auranofin and related gold(I) complexes as cytotoxic and anticancer drugs. We explore here whether AF may manifest any significant antiplasmodial effect and thus be of potential interest for malaria treatment.

Malaria is today a major health emergency on the international scale and also a real challenge for modern pharmacology [14]. Owing to the frequent appearance of drug-resistant *Plasmodium* strains, the classical antimalarial drugs tend to become rapidly obsolete, generating a continuous need for new therapeutic agents, possibly endowed with innovative mechanisms of action. Remarkably, *Plasmodium falciparum* is known to be particularly sensitive and vulnerable to oxidative stress; in particular, *P. falciparum* thioredoxin reductase has been validated as a "druggable" target for the development of new antimalarials [15]. As *P. falciparum* thioredoxin reductase bears a number of functionally relevant cysteines (but no selenol groups) [16], we thought that *thiol-reactive* gold drugs

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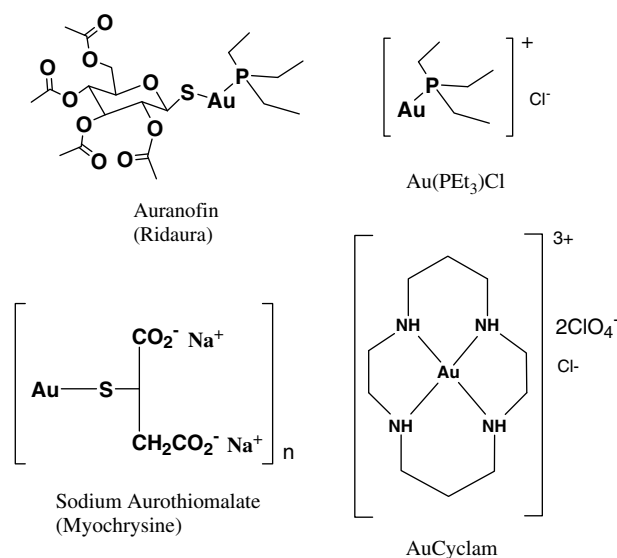E-mail address: luigi.messori@unifi.it (L. Messori).

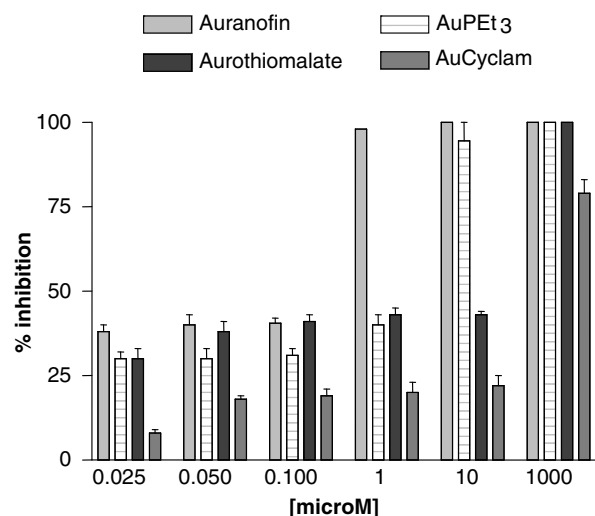Fig. 1. Chemical structures of the selected gold complexes.

Fig. 2. Antiplasmodial activities of gold compounds. Percentages of inhibition of 3D7 *P. falciparum* strain growth against increasing concentrations of auranofin, Au(PEt₃)Cl, aurothiomalate and AuCyclam, respectively. The data are expressed assuming no inhibition for untreated controls.

might display appreciable antiplasmodial actions, through a direct and potent inhibition of this enzyme. These observations prompted us to measure the effects of a few representative gold drugs on *P. falciparum* growth *in vitro*. To our surprise, despite a certain number of metal complexes, and even gold compounds, had earlier been tested as antimalarial agents [17], we realized that classical antiarthritic gold drugs, to the best of our knowledge, had never been considered for this scope.

3. Direct evidence that auranofin and a few related gold compounds strongly inhibit *P. falciparum* growth

The antimalarial effects of auranofin were assayed *in vitro* through a validated experimental procedure based on the determination of *P. falciparum* lactate dehydrogenase (pLDH) activity (see Supporting Information Available for details). Measuring pLDH provides indeed a selective and easy screening method for the identification and quantitation of parasite growth in *in vitro* cultures [18]. For comparison purposes the effects of three additional and structurally diverse gold compounds, namely gold(I) triethylphosphine chloride (Au(PEt₃)Cl), sodium aurothiomalate and gold(III) cyclam (AuCyclam), reported in Fig. 1, were measured as well. Au(PEt₃)Cl is a derivative of auranofin where the thioglucose ligand is replaced by a chloride ion; aurothiomalate is the antiarthritic gold(I) drug *myocrysine*; at variance, AuCyclam is a gold(III) compound where the gold center is tightly coordinated to four nitrogens, within a roughly square planar arrangement [19]. Such a strong coordination environment reasonably results into a marked decrease of reactivity.

Results of *P. falciparum* growth assays, in the presence of the various gold compounds, are shown in Fig. 2, while Table 1 summarizes the concentrations inhibiting the cell viability by 50% (IC₅₀) determined for each species. It is evident that auranofin, even at very low concentrations, causes a strong and nearly complete inhibition of *P. falciparum* growth, with an IC₅₀ value of 142 nM; Au(PEt₃)Cl shows a similar trend but with a significantly higher IC₅₀ value of. 2.1 μM. In contrast, aurothiomalate and AuCyclam are far less effective; their

Table 1
IC₅₀ values for auranofin, Au(PEt₃)Cl, aurothiomalate, AuCyclam, artemisinin (ART), ART/50 nM Auranofin and ART/100 nM auranofin against 3D7 *P. falciparum* strain

Name of compounds	3D7 *P. falciparum* strain IC₅₀ (95% CI)
Auranofin	142 nM (132–152)
Au(PEt₃)Cl	2.1 μM (2.0–2.2)
Aurothiomalate	168 μM (160–170)
AuCyclam	439 μM (428–440)
Artemisinin (ART)	16 nM (14.9–17.3)
ART/50 nM auranofin	12 nM[*] (8.9–12.3)
ART/100 nM auranofin	9 nM[*] (7–13)

[*]$P < 0.001$.

IC₅₀ values being 168 μM and 439 μM, respectively (Table 1). It is of interest to observe that all gold compounds, starting from the lowest tested concentrations, cause a partial and rather constant inhibition of *P. falciparum* growth (~10–25%); this observation implies, most likely, the coexistence of two independent cytotoxic pathways, one of which is saturated very soon. This peculiar behavior clearly emerges from inspection of the recorded *P. falciparum* growth inhibition profiles; characteristic profiles obtained in the case of AF and Au(PEt₃)Cl are reported in Fig. 3.

In our opinion, the above reported results are of great interest since a very important antiplasmodial activity is described for the first time for the antiarthritic gold(I) drug auranofin. This finding might imply that our working hypothesis is basically correct as the known and relevant inhibitory effects of AF toward thioredoxin reductase [9,10] are apparently reflected into a strong inhibition of *P. falciparum* growth.

Of course, further work is needed to confirm the validity of such hypothesis. In particular, specific studies on the inhibition of *P. falciparum* thioredoxin reductase by auranofin and related gold drugs would be warranted. In any case, if the relevant antiplasmodial effects that we have observed here *in vitro*, will be confirmed by the animal studies that we are planning, the way would be open for the rapid clinical testing
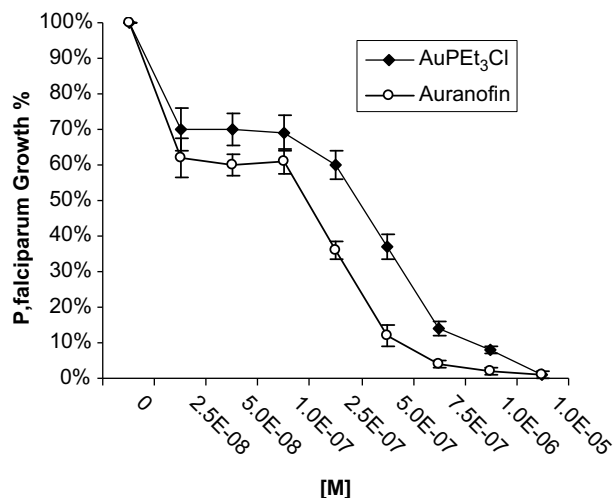
Fig. 3. Percentage of 3D7 *P. falciparum* growth as a function of the concentration (M) of auranofin or Au(PEt$_3$)Cl.

of auranofin in malaria treatment. Indeed, AF is already in the clinics since more than 25 years and presents a well known and quite safe toxicity profile: thus, its clinical use for a different therapeutic indication might be afforded very quickly and straightforwardly. Also, it is worthwhile reminding that AF is taken up very efficiently by erythrocytes [20], one of the major sites where a strong antiplasmodial action is required; this additional feature might render its *in vivo* antimalarial actions even more effective.

4. The combined effects of artemsinin and auranofin

Since we hypothesize that the specific antiproliferative effects of AF toward *P. falciparum* probably arise from induction of oxidative stress, we decided to measure its antiplasmodial effects in combination with artemisinin. Artemisinin (ART),
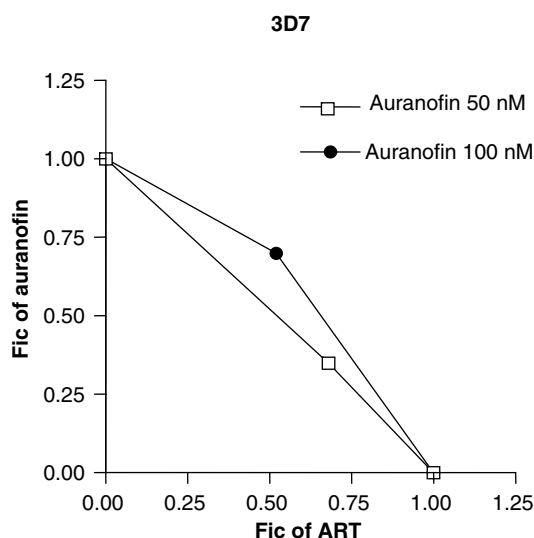

Fig. 4. Isobolograms showing the interaction between ART and auranofin when administered on 3D7 *P. falciparum* strain. ART was given at non-cytotoxic doses, ranging from 0.625 nM to 40 nM, in the presence of 0, 50 or 100 nM auranofin. Fic is the fractional inhibitory concentration.

today one of the most potent antimalarial agents available in the clinics (with an IC$_{50}$ value falling in the very low nanomolar range); is thought to work through induction of intracellular oxidative stress [21,22]. Thus, we wondered whether these two drugs, i.e. artemisinin and auranofin might manifest any significant synergism. Their possible interactions were evaluated through an established methodology relying on the so called "isobolografic" analysis [23,24]. Using AF, either 50 or 100 nM, in combination with artemisinin, the sum of FICs was 1.2 and 1.03, respectively, when administered on 3D7 *P. falciparum* strain. The results of isobolografic analysis are thus consistent with an *additive effect* of both auranofin concentrations, as shown in Fig. 4; any antagonism between these two agents may be ruled out.

5. Concluding remarks and perspectives

In conclusion, based on a simple mechanistic hypothesis and a few selective *P. falciparum* growth inhibition measurements *in vitro*, we have demonstrated for the first time that the antiarthritic drug AF displays very pronounced antiplasmodial effects, and thus merits further investigations as a potential antimalarial drug. Moreover, we have shown, through isobolografic analysis, that AF displays additive antimalarial effects when given in combination with artemisinin. Significant but less potent antiplasmodial effects have been measured for the other investigated gold compounds.

The observed antiplasmodial effects are probably mediated by severe oxidative stress originating from *P. falciparum* thioredoxin reductase inhibition. Of course, further experimental work is absolutely warranted to support this latter statement; however the presence of a few functionally relevant cysteine residues at the active site of *P. falciparum* TrxR makes us confident that our working hypothesis is well grounded. If the effectiveness of antimalarial drug discovery strategies based on direct inhibition of thioredoxin reductase by metallic compounds will be eventually proved for AF, the way is open to the extensive testing of several other *thiol reactive* metallodrugs showing acceptable toxicity requirements.

Finally, we like to stress further that AF is in clinical use since more than 20 years. Looking for new uses of old (and thus *clinically established*) drugs represents today a very promising and effective strategy of modern drug discovery with obvious advantages. Indeed, much work concerning the safety profile can be avoided leading to a drastic reduction of times and costs. New therapeutic applications for some established drugs have been identified in the last years; a few relevant cases are described in the recent literature [25,26]. Auranofin might hopefully represent another successful example for this kind of strategy.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.02.028.

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