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Review

On the Action of N^1, N^1-Anhydro-bis(β-hydroxyethyl)-Biguanide Hydrochloride (Abitylguanide, Moroxidine, ABOB) Versus RNA Viruses

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Abstract

The review considers the compound N^1, N^1-anhydro-bis(β-hydroxyethyl)-biguanide hydrochloride (abitylguanide, ABOB) as the first applied in the clinical practice anti-flu drug, which later did not prove efficacious, was officially rejected and classified as an agent whose merit only deserved a place in the history of antivirals. The compound manifested some activity against other RNA-containing viruses (paramyxo) but with contradictory effects, strongly dependent on the test methods used and on the virus inoculation dose (multiplicity of infection).

Key words: abitylguanide, influenza viruses, paramyxoviruses

Резюме

Обзорът разглежда съединението N^1, N^1-anhydro-bis(β-hydroxyethyl)-biguanide hydrochloride (abitylguanide, ABOB), първият приложен в лечебната практика антигрипен препарат, който по-късно не доказа своята ефикасност, бе официлно отхвърлен и класифициран като средство, имащо място само в историята на антивиралите. Съединението показа известна активност спрямо други РНК вируси (парамиксо), но с противоречиви ефекти, силно зависими от приложения тест метод и от вирусната инокулационна доза (множественост на инфекцията).

Concerning the anti-influenza action

In 1960, Bo Melander announced that the compound designated by the generic name abitylguanide (moroxidine) or by the abbreviation ABOB, manifested a protective effect against influenza pneumonia in mice, infected via inhaling influenza viruses A/Puerto Rico/8 (H1N1) and B/Lee, expressed by a reduction of the degree of both pathologic changes and virus titer in the lungs. This marked the start of intensive research on the anti-influenza activity of this substance and of the biguanide derivatives in general during a period when antiviral chemotherapy was making its first steps. It was established that ABOB possessed some activity on the replication of influenza viruses A(H1N1), A(H2N2) and B *in vitro* (Rada, 1962), *in ovo* (Liu and Engle,1960; Goret and Pilet, 1963) and *in vivo* (Melander, 1960a; Goret and Pilet, 1963).

In spite of its moderate antiviral effect in experimental conditions (according to contemporary criteria), in the absence at that time of other anti-influenza substances and proved to be absolutely harmless (Söberg, 1960a; Cutting, 1962), ABOB comparatively quickly was included in clinical trials in influenza infections, including via the double-blind study scheme, organized by the Swedish company AB/KABI (Stockholm). In those trials the compound was applied in tableted combination with small doses of methatropine nitrate and methscopolamine nitrate, named Flumidin (AB/KABI). By the end of 1961, Flumidin [Virustat (Delagrange, France), Virugon, Virunil, Virusmin, Spenitol, Influmin (Polfa, Poland) and other trade names] was tested on as many as 15,000 persons with influenza A(H1N1), A(H2N2) and B, demonstrating, according to the judgment of a series of researchers (Söberg, 1960b; Almberg, 1960; Melander, 1960b, 1963; Jordan, 1961; Sandring, 1962; Zetterberg *et al.*, 1962; Wheatley, 1963; Haglind, 1964; Kitamoto, 1964; Klettenhammer, 1964), a generally favorable, although moderate, effect at prophylactic administration (in tablets of 300-500 mg twice daily), evidenced by a certain decrease in the incidence of influenza. When applied in a ther-

apeutic dose of 300-500 mg thrice daily for 7-14 days starting on the first days of the symptomatic appearance, a mild course of the disease was observed (Söberg, 1960a,b; Kitamoto, 1964; Haglind, 1964; Klettenhammer, 1964). In the meantime, reports appeared emphasizing the value of Flumidin as a prophylactic means, which called into question its therapeutic effect (Söberg, 1960a, b; Dumon *et al.*, 1963; Haglind, 1964), while other reports completely rejected its efficacy (Parker *et al.,* 1962; Jackson, 1963; Stanley *et al.,* 1965).

Whereas Parker *et al.* (1962) considered Flumidin use in viral infections of the upper respiratory tract of unspecified etiology, the double-blind studies carried out by Jackson (1963) and Stanley *et al.* (1965) merited serious attention. The completely negative results of these very well controlled trials proving the inefficacy of ABOB in influenza gave a grounding of the Standing Joint Committee on the Classification of Proprietary Preparations of Great Britain to place this medicament in the category of unacceptable drugs, as a drug with improved value (1967) (Bauer, 1972). Despite the data about a certain prophylactic effect of ABOB combined with homatropin-methylbromide and vitamin C (Morgalin, Chinoin, Hungary) in influenza infection in a closed children group (Ambró and Nagy, 1974), monographies and publications on antiviral substances after 1970 passed over in silence this substance or pointed it as an example of non-effective anti-influenza drug (Bauer, 1972; Sidwell and Witkovski, 1979; Zlydnikov *et al.,* 1979). Zlydnikov *et al.* (1979) classified ABOB within the pathogenetic and symptomatic action in influenza.

The analysis of the results of experimental *in vitro* and *in vivo* studies carried out with ABOB, estimated according to the contemporary criteria for antiviral activity, demonstrates that the involvement of this compound in clinical trials (and even in *in vivo* tests) for influenza has been groundless.

We have carried out tests to compare the effect of ABOB and other anti-influenza antivirals – rimantadine hydrochloride, ribavirin and mopyridone on the model of the orthomyxovirus - influenza virus A/chicken/Germany/27 (FPV, Weybridge) (H7N7) by the plaque-reduction method and demonstrated that ABOB did not possess a marked inhibitory effect (Galabov *et al*., 1981).

Effect towards paramyxoviruses and other RNA viruses

In 1964, we established that ABOB tested against the two antigenic variants of paramayxovirus type 1 Sendai, Kuroya strain (Japanese variant) and 960 strain (Vladivostok variant), at non-toxic concentrations (375 – 1100 μg/ml) manifested some inhibitory effect on the replication of this virus in calf kidney primary cell cultures. On the basis of its value, this effect could be classified as a moderate to a weak one, being strongly dependent on the multiplicity of infection (m.o.i.). A study on the mode of action of the compound emphasized the role of suppression of viral protein synthesis (characterized via the direct immunofluorescent method) (Galabov, 1966, 1968a, 1968b). ABOB manifested a marked suppression effect on the virus growth in embryonated hen eggs (Galabov, 1966).

In the meantime, Rada and Závada (1962), using their own developed agar-diffusion plaque-inhibition method, reported that ABOB was ineffective against another paramyxovirus, Newcastle disease virus. In contrast, Gherganov *et al.* (1986) established a pronounced antiviral effect of the compound against strain P of this virus, using a novel *in vitro* test system, tracheal organ cultures of pheasants. A complete protection of the ciliary activity (at 150-1500 50% cilliostatic doses) under ABOB was registered.

ABOB was inactive against togavirus Western equine encephalomyelitis virus in the agar-diffusion plaque-inhibition test (Rada and Závada, 1962), against poliovirus 1 (the Sabin's LSc-2ab vaccinal strain and the virulent Mahoney strain) (Galabov, 1978) in one-step growth cycle experimental design in HeLa (S_3 clone) and KB cells, respectively, and against vesicular stomatitis virus replication in L cells (Galabov, 1978). Some inhibitory effect was registered against bovine leukemia virus reproduction in FLK cells, but not against Mc-29 avian leukemia virus (Galabova and Galabov, 1983).

In conclusion,

the biguanide ABOB activity on the replication of RNA-containing viruses could be considered as a weak one, with a contradictory effectivity, the selectivity ratio values being markedly below the requirements of the established contemporary methodological criteria for antiviral substances. The pioneer role of this substance as an anti-flu agent did not meet the requirements of the anti-flu experimental chemotherapy, when the compound was studied by contemporary methodical tools. Therefore, ABOB only remained as an ineffective anti-influenza drug in the history of this research area.

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